VOL. 2 FASC. 4

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(Postverlagsort Berlin · 9. 8. 1961)

Die Forschung über die Wirkungen und Wirkungsweisen psychotroper Substanzen hat in den letzten Jahren einen unerhörten Aufschwung genommen. Was vordem nur ein erwünschtes Ziel war, ist zu einer neuen Wissenschaft geworden: Psychopharmakologie. Da eine fruchtbare Analyse und Synthese ihrer Probleme nur durch Zusammenarbeit aller Grundfächer (Pharmakologie, Neurochemie, Neurophysiologie, Neurologie, Psychologie und Psychiatrie) möglich wird, ist die Psychopharmakologie eine verbindende, integrierende Forschungsdisziplin. Die ständig anwachsende Literatur dieses komplexen Arbeitsgebietes ist jedoch bisher zwangsläufig über zahlreiche Zeitschriften verstreut, da es bis heute kein Spezialorgan gab, das sich ausschließlich der Psychopharmakologie widmet. Diesem dringenden Bedürfnis zu begegnen, hat sich eine Gruppe von Vertretern der verschiedenen Arbeitsrichtungen der Psychopharmakologie entschlossen, eine neue Zeitschrift „Psychopharmacologia" zu gründen. In ihr sollen die bedeutenden Fortschritte dieses Arbeitsgebietes durch Veröffentlichung experimenteller und klinischer Originalarbeiten, Übersichten der neuesten Literatur sowie kurzer Originalmitteilungen zusammengefaßt werden.

Recent years have witnessed an unprecedented advance in research on the action and effects of psychotropic drugs, and what, formerly, was just a distant goal, has now evolved into a new branch of science: psychopharmacology. As, however, any fruitful analysis and synthesis of its problems can only be attained with the aid of the complete scale of basic sciences (pharmacology, neurochemistry, neurophysiology, neurology, psychology and psychiatry), psychopharmacology constitutes an integrating discipline of research. Owing to the lack of an organ devoted especially to psychopharmacology, the constantly increasing literature pertaining to this complex field of activity has hitherto of necessity been scattered among various periodicals. In order to overcome this drawback, a group of representatives of the various psychopharmacologic sections have engaged in editing a journal, "Psychopharmacologia", in which the publication of original experimental and clinical papers, reviews of recent literature and short original notices will provide a comprehensive survey of the important progress which is being actually achieved in this field of science.

Ces dernières années ont vu un développement sans précédent dans la recherche des effets et du mode d'action des substances psychotropes sur le «Comportement» et ont fait naître une nouvelle science: la Psychopharmacologie. Comme ces problèmes ne peuvent être résolus que par la collaboration des disciplines de base telles que la pharmacologie, la neurochimie, la neurophysiologie, la psychologie et la psychiatrie, la psychopharmacologie est devenue un champ de recherche de première importance. Cependant la littérature toujours croissante en ce domaine de recherche est forcément disséminée dans de nombreux périodiques, puisqu'il n'existe pas encore de journal exclusivement consacré à la psychopharmacologie. Pour répondre à ce pressant besoin un groupe de représentants des diverses disciplines de la psychopharmacologie s'est mis en devoir de rédiger un nouveau journal dans lequel seraient rassemblés les progrès importants de ce domaine, par la publication d'ouvrages originaux expérimentaux et cliniques, ainsi que des rapports sur des questions actuelles.

Richtlinien für die Mitarbeiter siehe am Schluß des Heftes. — Directions to Authors are given at the end of this number. — Directives destinées aux auteurs, voir à la fin du fascicule.

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Psychopharmacologia

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Der Erlebniswandel des Schizophrenen

Ein psychopathologischer Beitrag
zur Psychonomie schizophrener Grundsituationen

Von Dr. med. Dr. phil. K. P. KISKER,
Privatdozent für Psychiatrie und Neurologie an der Universität Heidelberg

Mit einem Geleitwort von Prof. W. v. BAEYER, Heidelberg

(Monographien aus dem Gesamtgebiete der Neurologie und Psychiatrie. Heft 89)

Mit 9 Abbildungen. VIII, 140 Seiten Gr.-8°. 1960. DM 29,80

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Einleitung · Zugänge zur schizophrenen Person: Philosophisches Vorverstehen des verrückten Daseins. Anthropologische Psychopathologie als begegnendes Verstehen. Dynamische Topologie als Beispiel eines konstruktiv-entwerfenden Verständnisses · Wandlungen der Selbstverfügbarkeit des Schizophrenen: Vorfeld. Auseinandersetzung. Einordnung. Ausgliederung · Zur Theorie des schizophrenen Personwandels: Inkompatibilität als dynamisches Prinzip. Strukturwandel als übergreifendes Prinzip · Der Forderungscharakter des schizophrenen Erlebniswandels: ein Teilproblem der Psychotherapie der Verrücktheit · Literatur · Namen- und Sachverzeichnis.

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1 Harris, H.: J. Amer. Med. Ass. **172**, 11, 1162 (1960)

2 Voelkel, A.: Vortrag Tagg. Dtsch. Sektion Internat. Ges. Psycho-Pharmako-Therapie Nürnberg 30. IV. - 1. V. 1960

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Psychopharmacologia 2, 221—223 (1961)

W. Mayer-Gross

15. 1. 1889—14. 2. 1961

Professor MAYER-GROSS, who died at his home in Birmingham on February 14th this year, had been a member of the Editorial Board of this Journal since its inception. He will be remembered not only for his outstanding contribution to clinical psychiatry but also for his interest in and encouragement of research in various fields. The importance he attached to psychopharmacology is reflected in his work on experimental psychosis produced by hypoglycaemia and hallucinogenic drugs and in the part he played in the founding of a journal devoted to this field of study. He was also a founder member of the Collegium for Neuro-Psychopharmacology and took an active part in its affairs.

Born in Germany, MAYER-GROSS spent the early of his career in Heidelberg where he graduated in medicine. He went to England in 1933 and remained there for the rest of his life, working first at the Maudsley Hospital, then at the Crichton Royal, Dumfries and for the last six years in Birmingham, where he was Senior Fellow in the Department of Experimental Psychiatry at the School and Director of Clinical Research at the Uffculme Clinic. At the time of his death he had retired from these posts and was about to return to Heidelberg where his property and University post had been restored to him.

P. B. BRADLEY

It will take time for those who worked with, or were taught by, WILLIAM MAYER-GROSS to grasp that he is gone. His life was as rich as his person; and many were the friends and acquaintances from all parts of the world who passed through his study, or enjoyed the hospitality of his home. He was one of those rare human beings who are all of a piece. His psychiatry sprang from his humanity, and his bent as an investigator from his ever-present curiosity. He wanted to know, and he wanted to help; and there was little he allowed to get in the way of knowing, and of helping.

This is not the place to trace at any length — for such length would be required — the path which led WILLY MAYER-GROSS to the position he occupied in British and World Psychiatry. That his professional life should have begun in one University — Heidelberg (where he was appointed Privatdozent in 1924, and elected Pro-

fessor Extraordinarius in 1929) and ended in another — Birmingham (where, as noted, he was elected Senior Fellow in 1954), is as appropriate as that a great and important period of his life should have been spent in two mental hospitals, the Maudsley (1934 to 1939) and the Crichton Royal, Dumfries (1939—1954). It is this mixture of the academic and the clinical, and the fusion of both into a kind of practical knowing which made him the remarkable person he became. The view of psychiatry which he held, with its consistent emphasis on phenomena, and the description of phenomena, is one which is neither popular, nor judged novel in our day. Yet it may well be the interaction of this view with testable psychophysiological, psychodynamic and psychosocial theories which will further the evolution of Psychiatry as a Clinical Science. MAYER-GROSS' early writings had uncommon breadth, range and solidity. His contribution to the volume on Schizophrenia in Bumke's Handbook remains a landmark to this day — prodigious in the richness and depth of its descriptive material. His papers on oneirophrenia and the organic brain syndromes are standard teaching texts. Readers of this Journal will also know how early, and with what single-mindedness, MAYER-GROSS took up the study of psychotropic drugs. A paper on Cocain dates from 1920; and there followed from Germany, and from England a steady series of contributions on Mescalin, on the hypoglycaemic state, and on the effects of LSD-25, spread evenly over the years. With it, and in parallel, there was always a keen interest in Psychiatry as a whole: There were epidemiological studies in Dumfries and in Birmingham; and there was, in the closing phase of his life, the experiment in Social Psychiatry at the Uffculme Psychiatric Early Treatment Centre of the Birmingham Regional Hospital Board and Birmingham University.

Two journals and two major psychiatric texts have felt his sustained participation. He played a leading part in the foundation of the 'Nervenarzt' and, as already noted, of 'Psychopharmacologia'. His 'Clinical Psychiatry', published together with ROTH and SLATER, is now being translated into several languages; and as an Editor of 'Psychiatrie der Gegenwart' (Volume II of which has already appeared), he and his colleagues worked on this recent, broad, comprehensive presentation of the subject. He served as Consultant to the World Health Organization in drawing up plans for the All India Institute of Postgraduate Psychiatric Research and Training; he was a past president of the Section of Psychiatry of the Royal Society of Medicine; and, in 1958, delivered the Adolf Meyer Lecture before the American Psychiatric Association.

Somehow when I think of WILLY MAYER-GROSS I think of a gay, bouncing, solid, hard rubber ball. Up in the air, and down to earth and, withall, completely indestructible. I well remember the time when he joined a harassed new Executive Head in the Department of Experimental Psychiatry in Birmingham seven years ago. We were building Uffculme Clinic then, and many were the paths we had to travel. On a particularly gloomy morning, there would be a knock on the door; then it would open slowly; and through the crack there would appear a nose — a sort of rock of a nose, around which the rest of the face was built; then a pair of bright, kind eyes, glistening behind gold rimmed spectacles; and lastly an enormous ear-to-ear grin. "Don't take it serious", he would say, and disappear. And one would laugh, and forget, and feel better for the fact that WILLY had come in and was next door.

Today this journal looks into the future of a new science. It is good to recall one who looked early, courageously, and well. A man completely devoid of guile or malice; a man at once strong, warm and gay. A rare man, indeed.

JOEL ELKES

Psychopharmacologia 2, 224—242 (1961)

Original Investigations · Originalarbeiten · Travaux originaux

Institute of Pharmacology, Medical University, Pécs (Hungary)

Further Studies on the Metabolic Background of Tranquilizing Drug Action

By

L. Decsi

(Received July 20, 1960)

The experiments to be reported started with the observation that compounds with chlorpromazine-like central nervous action[1] exert two characteristic effects on brain metabolism: all derivatives so far investigated inhibit both the oxidative phosphorylation (OP) and the adenosine triphosphatase (ATPase) activity of brain cells, i.e., they influence energy production as well as energy utilization (Decsi 1957; Decsi and Nádor; Decsi 1958a). These inhibitory effects seem to be closely related to the pharmacological action since i. compounds inhibiting either OP or ATPase alone are devoid of chlorpromazine-like action *in vivo* (Decsi 1958a; 1958b); ii. the potency *in vivo* of a compound can be predicted on some tests with sufficient accuracy merely from biochemical data (Decsi 1958a, 1958b).

The close quantitative relationship between enzyme-inhibitory effects *in vitro* and pharmacological activity *in vivo* was demonstrated for the ether-anaesthesia prolonging effect and, with a great number of compounds not included in the present study, for the sedative action too. By extending our investigations to other drugs and by using two new methods *in vivo* (blocking of conditioned reflexes and prolongation of the hexobarbital anaesthesia), a detailed analysis was made of this relationship in the experiments hereon reported.

Methods

Tests in vivo. *1. Conditioned reflex.* A conditioned avoidance response in the rat was developed according to the procedure generally employed. The unconditioned stimulus was an electric shock (60 V) from the floor of the apparatus, while the conditioned one was a bell. Inhibition of the response was judged on the basis of the "all-or-none" principle, considering the reaction inhibited when the animal

[1] For sake of brevity and being fully aware of the limitations of such a generalization, this expression was chosen to design compounds having anaesthesia-prolonging action, decreasing spontaneous motor activity, antagonizing central nervous stimulants, eliciting motor incoordination and blocking conditioned reflexes in animal experiments, *and*—when studied—being regarded tranquilizers in the clinic irrespective of the finer characterization of the drug action (major or minor tranquilizer, etc.).

failed to produce the conditioned reflex (jumping up to an isolated stand mounted 30 cm over the floor) within 5 seconds following the bell. Normal reflex latency time of untreated rats lay between 1.2 and 1.6 sec.

The drugs were dissolved in physiological saline, or—in the case of meprobamate and chlorazacyclonol—in propylene glycol subsequently diluted with saline. Propylene glycol itself in the amounts used (20 to 40 mg/100 g) had no influence on the reaction. After measuring the control latency time, drugs were administered intraperitoneally and the response was reinvestigated 1, 2 and 3 hours following the injection. In order to eliminate any possible difference in the absorption rate, the effectiveness of a drug was judged by the maximum inhibition produced during experimentation, irrespective of the time, at which it occured.

2. Potentiation of the effect of hexobarbital. Mice were pretreated intraperitoneally with the drug under investigation, and 30 min later, 30 mg/kg of hexobarbital (in saline) were injected intraperitoneally. This dose of hexobarbital is subanaesthetic, as no animal treated at this dose level showed anaesthesia. The hexobarbital-effect was considered potentiated when within the first 15 min following hexobarbital the mouse remained in side position for at least 60 sec. Quantitative evaluation was made on this test as follows: the drug under investigation was administered at 2 or 3 dose levels, each dose to 10 to 20 mice, and the number of animals anaesthetized was registered. From the dose-response curves obtained in this manner, the AD_{50} value (dose producing anaesthesia in 50 per cent of the animals) was then computed. As a rule, the compounds were dissolved in physiological saline and injected in a volume of 0,1 ml/10 g.

Tests in vitro. *1. Oxidative phosphorylation* was measured on rat and dog brain homogenates, according to the method already described (DECSI and NÁDOR; DECSI and MÉHES 1959).

2. Adenosine triphosphatase activity was measured on rat brain homogenates. The method of DU BOIS and POTTER was employed, using Mg^{++} as activating ion and veronal-HCl as buffer. (For details see DECSI and MÉHES 1957). The inorganic phosphate liberated was determined after precipitation with calcium (FISKE and SUBBAROW). As a rule, enzyme activity was measured at pH 7,4. In some cases, however, the experiments were performed at pH 6,4, due to the poor solubility at pH 7.4 of certain drugs (e.g. promethazine).

Results

I.

Table 1 shows the effect of the drugs investigated on the conditioned reflex responses. Highest activity was found with compound P-824 (N-methyl-piperidyl-(4)-methyl-phenothiazine, NIESCHULZ et al. 1956), lowest activity with hydroxyzine and meprobamate. It is to be noted that the block of the conditioned reflexes observed after the highest dose of meprobamate (200 mg/kg) was unspecific in character, i.e., in most animals the unconditioned response was similarly inhibited. At the dose levels used, all other compounds produced specific block only.

The effects of benactyzine on conditioned reflexes was uncertain. A dose of 30 mg/kg produced inhibition over 60 per cent on the one day, but only 10 per cent on the other. Irregular results with benactyzine were observed also if the drug was administered on the same day at various dose levels: 20 mg/kg elicited 50 per cent inhibition in one group of rats, while 40 mg/kg produced only 30 per cent block in

Table 1. *Influence on the conditioned reflex response of the rat*

Compound	N	ED_{50} mg/kg	95 per cent fiducial limit	Slope*	Relative activity
Chlorpromazine . . .	60	3.47	2.96—4.41	1.77	1.0
P-824	40	2.82	1.94—3.92	1.80	1.23
Promazine	20	5.89	3.64—9.54	1.62	0.60
Propionylpromazine .	20	5.0	2.25—11.28	3.68	0.70
Mepazine	40	11.72	7.12—19.3	1.65	0.30
RP 3300	40	17.0	12.5—23.1	1.64	0.20
Promethazine	40	28.2	21.6—38.2	1.60	0.12
Azacyclonol	40	43.0	33.2—54.1	1.42	0.08
Chlorazacyclonol** .	20	37.1	23.5—58.6	1.56	0.11
Meprobamate	60	72.5	51.4—98.0	1.92	0.05
Hydroxyzine	40	100.0	55.6—180.0	2.58	0.03

* With 95 per cent probability, all curves except that of propionylpromazine run in parallel to each other.

** M.G. 3088 (s. CAVALLINI et al.).

Table 2. *Anaesthesia-prolonging action in the mouse*

Compound	N	AD_{50} mg/kg	95 per cent fiducial limit	Slope*	Relative activity
Chlorpromazine . . .	50	3.09	2.44—3.92	1.80	1.0
P-824	20	3.51	2.72—4.53	1.49	0.88
Promazine	20	8.02	5.08—12.82	2.19	0.39
Propionylpromazine .	30	4.26	3.11—5.85	1.68	0.73
Mepazine	20	25.0	7.8—80.0	2.28	0.12
RP 3300	20	23.7	19.0—29.6	1.44	0.13
Promethazine	20	12.46	8.14—18.0	2.03	0.25
Azacyclonol	30	37.2	18.2—76.4	3.24	0.08
Chlorazacyclonol . . .	30	33.1	24.6—44.7	1.64	0.09
Meprobamate	20	85.0	59.5—121.8	1.79	0.04
Hydroxyzine	20	47.2	31.2—71.2	1.95	0.07
Benactyzine	20	19.7	12.1—32.2	2.24	0.16

* With 95 per cent probability, all curves run parallel to each other.

the other. This peculiar response to benactyzine was also observed by MAFFII. As a consequence, the potency of benactyzine as a blocker of the conditioned reflex could not be determined.

Potentiation of the action of hexobarbital by these drugs is shown in Table 2. Chlorpromazine chosen for comparison proved the most active drug, causing 50 per cent anaesthesia (AD_{50}) in a dose of 3.09 mg/kg. Meprobamate exhibited the slighest activity on this test (AD_{50} = 85 mg/kg).

The next step in our study was to investigate the effect of the drugs on *brain metabolism*. Influence on OP is shown in Table 3. As seen, phenothiazine derivatives are roughly identical in inhibitory potency, while the uncoupling effects of meprobamate, azacyclonol, chloraza-

cyclonol and benactyzine are much weaker than that of chlorpromazine. Table 3 shows that *all compounds inhibit oxidative phosphorylation.*

In previous experiments we have demonstrated that the uncoupling effects of chlorpromazine, meprobamate and benactyzine were not of the same strength in various brain areas (DECSI and MÉHES 1959; DECSI and MÉHES 1958). The hypothalamus exhibited much greater sensitivity than did other parts of the brain. These experiments were made on dog and, in some cases, on cat brain, and they are now extended to mepazine, P-824 and azacyclonol. As seen from Table 4, the uncoupling effects of the drugs are more marked on hypothalamic than on cortical cells, just as those of meprobamate, chlorpromazine and benactyzine. *This increased sensitivity of the hypothalamic cells seems to be characteristic of the tranquilizing drugs.*

Table 3. *Inhibition of the oxidative phosphorylation*

Compound	N	ID_{50} $\times 10^{-4}$ M	Relative activity
Chlorpromazine	52	1.59	1.0
P-824	24	1.64	0.97
Promazine . .	15	2.04	0.78
Propionyl-promazine .	20	2.04	0.78
Mepazine . . .	22	2.82	0.56
RP 3300 . . .	21	2.36	0.67
Promethazine .	26	2.02	0.79
Perphenazine .	17	1.0	1.59
Azacyclonol . .	17	15.1	0.11
Chlorazacyclonol	23	5.96	0.27
Meprobamate .	20	18.2	0.09
Hydroxyzine .	12	6.45	0.25
Benactyzine . .	24	2.48	0.64

The effect of the drugs on *ATPase* in shown in Table 5. Highest inhibitory activity was exerted by perphenazine, while hydroxyzine proved least active. Form the data of Table 5 the conclusion can be drawn that *every compound inhibits ATPase activity.*

Table 4. *Effect of Mepazine, P-824 and Azacyclonol on the oxidative phosphorylation of the cerebral cortex and hypothalamus in the dog*

Compound	Concentration	Inorg. P. uptake μM/g/h					
		Cerebral cortex			Hypothalamus		
		N	Control	Treated	N	Control	Treated
Mepazine .	$1 \cdot 10^{-4}$ M	6	253.0	245.0 *— 3.2%*	6	288.0	241.0 *— 16.5%*
P-824 . . .	$1 \cdot 10^{-4}$ M	12	288.0	283.0 *— 1.6%*	12	244.0	163.9 *— 32.9%*
Azacyclonol	$1 \cdot 10^{-3}$ M	6	271.0	241.0 *— 11.0%*	12	216.0	131.8 *— 39.3%*

In further experiments an attempt was made to compare quantitatively the effect on brain metabolism *in vitro* and the pharmacological action *in vivo*. All compounds inhibit both OP and ATPase. In previous experiments we have found that i. the pharmacological activity depended

on the strenght of *both* inhibitory actions, and ii. the relative pharmacological potency was given by the product of the relative strengths of the enzyme-inhibitory actions (DECSI 1958a, 1958b). The results of this

Table 5. *Inhibition of the adenosine triphosphatase activity*

Compound	N	$ID_{25} \times 10^{-4}$ M*	Relative activity	Compound	N	$ID_{25} \times 10^{-4}$ M*	Relative activity
Chlorpromazine	44	2,11	1,0	Perphenazine .	18	0,59	3,54
P-824	26	2,69	0,79	Azacyclonol . .	16	5,12	0,41
Promazine . .	12	3,02	0,70	Chlorazacyclonol	34	4,17	0,51
Propionylpromazine .	16	3,80	0,56	Meprobamate .	30	10,7	0,20
Mepazine . . .	28	6,6	0,32	Hydroxyzine .	16	16,6	0,13
RP 3300 . . .	27	5,25	0,40	Benactyzine . .	38	5,0	0,42
Promethazine .	21	8,8	0,24				

* Owing to the pecularities of the enzyme reaction (WOLLEMANN and FEUER), the ID_{25} value rather than the ID_{50} value was used.

Table 6. *Prediction of the relative potencies in vivo from data of experiments in vitro*

Compound	Relative inhibitory potency		Calculated activity in vivo (A×B)	Potency in vivo			
				Block of cond. responses		Anaesth. prol.	
	Oxid. phosph. (A)	ATPase (B)		Rel. activity	95 per cent fiducial limit	Rel. activity	95 per cent fiducial limit
Chlorpromazine	1.0	1.0	1.0	1.0		1.0	
P-824	0.97	0.79	0.78	1.23	0,63—2,41	0.88	0.62—1.26
Promazine . .	0.78	0.70	0.55	0.60	0,35—1,03	0.39	0.22—0.68
Propionylpromazine	0.78	0.56	0.44	0.70	0.17—2.86	0.73	0.50—1.07
Mepazine . .	0.56	0.32	0.18	0-30	0.17—0.54	0.12	0.04—0.41
RP 3300 . .	0.67	0.40	0.27	0.20	0.11—0.35	0.13	0.09—0.17
Promethazine	0.79	0.24	0.19	0.12	0.07—0.21	0.25	0.15—0.42
Perphenazine	1.59	3.54	5.63	*			
Azacyclonol .	0.11	0.41	0.05	0.08	0.05—0.13	0.08	0.04—0.17
Chlorazacyclonol	0.27	0.51	0.14	0.11	0.07—0.18	0.09	0.06—0.13
Meprobamate	0.09	0.20	0.02	0.05	0.03—0.05	0.04	0.02—0.06
Hydroxyzine	0.25	0,13	0.03	0.03	0.01—0.08	0.07	0.04—0.11
Benactyzine	0.64	0.42	0.27	—	—	0.16	0.09—0.28

* Due to the small amount of drug available, no in vivo experiments could be performed.

calculation are shown in Table 6. As seen, *when chlorpromazine = 1, the mean value of the potencies found in vivo is 0.36, while that of the potencies calculated theoretically is 0.33.* The difference is, thus, less than 10 per cent, a fact idicative of the practical indentity of both series of values.

II.

In his review article on the role played by the uncoupling effect in the pharmacological action, BRODY put forward three criteria to be fulfilled for bringing a biochemical effect into causal relationship with a pharmacological action. These criteria are as follows:

1. The concentration necessary to produce the action *in vitro* should be comparable to that occuring *in vivo*.

2. When a drug acts *in vivo* primarly on one specific tissue, the effect *in vitro* should be more marked on that than on any other tissue.

3. Among structurally related drugs, a close parallelism should exist between enzyme-inhibitory action *in vitro* and pharmacological effect *in vivo*. Compounds, which are active *in vivo* should be also active on the biochemical level, while derivatives without effect *in vivo* should not have any action *in vitro*.

Considering our findings in the light of these criteria, we have the following picture:

ad 1. After giving 5 mg/kg chlorpromazine, GOUZON et al. found in the brain 13.1 μg/g = $3.7 \cdot 10^{-5}$ M drug. The brain level after 20 mg/kg was 84.5 μg/g = $2.38 \cdot 10^{-4}$ M (SALZMAN and BRODIE). The distribution of chlorpromazine within the brain is not equal: hypothalamus contains four times as much of the drug as do other brain parts (WASE et al.). This means that the concentration *in vivo* of chlorpromazine must reach, or even exceed, the level causing very strong inhibition of both enzyme reactions.

ad 2. Tranquilizing drugs act on brain, particularly on subcortex (hypothalamus and reticular formation). In a previous paper we have demonstrated that the brain cells are much more sensitive to the uncoupling effect of tranquilizing drugs than are liver cells (DECSI and MÉHES 1959). In addition, a difference in sensitivity was found also within different regions of the brain: concentrations causing marked uncoupling in the cells of the hypothalamus were ineffective in other parts of the brain. This increased sensitivity of the hypothalamic cells was demonstrated for chlorpromazine (DECSI and MÉHES 1958), meprobamate, benactyzine (DECSI and MÉHES 1959) as well as for mepazine, P-824 and azacyclonol (present experiments).

ad 3. As to the relationship between enzyme-inhibitory effect *in vitro* and pharmacological action *in vivo*, it was demonstrated for a great number of compounds that a chlorpromazine-like action *in vivo* was given only by derivatives inhibiting both enzyme reactions. Inhibition of OP alone or of ATPase alone was never coupled with a measurable pharmacological action, but the presence of both inhibitory effects was in all cases linked with the appearance of chlorpromazine-like activity *in vivo* (DECSI 1958b).

In addition ,we have demonstrated with an other series of drugs that prolongation of ether anaesthesia as well as the sedative action was related qualitatively and quantitatively to the enzyme inhibitory effects (DECSI 1958a, 1958b). In the present paper this quantitative relationship between the effects *in vivo* and *in vitro* was demonstrated for the block of conditioned reflexes and for the potentiation of the anaesthetic action of hexobarbital. Thus, not only the fulfillment of the three criteria of BRODY, but the quantitative relationship, which was found, indicates a high correlation between the pharmacological action of these drugs and the two biochemical effects described.

III.

The statement just made should also be examined in the light of the data published in the literature. The question to be answered is: do the relative pharmacological potencies, calculated theoretically from biochemical data, correspond to the potencies found in the experiments of other authors ? Throughout these calculations, chlorpromazine serves as the standard for comparison, its potency being the reference value = 1. (Results in Table 7.)

1. Promethazine. In the experiments of COURVOISIER et al. the spontaneous motility of mice was inhibited five times more by chlorpromazine than by promethazine. The potency of the latter is, thus, *0.20*. COURVOISIER investigated also the effects of these drugs on the conditioned reflexes of the rat. In this test, promethazine proved ten times weaker than chlorpromazine, which corresponds to an activity of *0.10*. According to TEDESCHI et al. (1958), a 50 per cent inhibition of the spontaneous motor activity of mice was elicited by 4.7 mg/kg chlorpromazine and 106 mg/kg promethazine, an experiment showing a relative potency of *0.04*. The sedative effect of chlorpromazine and promethazine on morphine-excited mice was compared by KOUZMANOFF et al. The ED_{50} value was 1.4 mg/kg with chlorpromazine and 9.4 mg/kg with promethazine. The relative potency of the latter drug is, thus *0.15*. The mean effective dose of chlorpromazine on "souris tournantes" is 2 mg/kg, while that of promethazine is 10 to 15 mg/kg (THUILLIER and NAKAJIMA). From these data a relative activity of *0.13 to 0.20* can be calculated for promethazine. According to VIAUD, 20 mg/kg chlorpromazine prolongs barbiturate anaesthesia by 1250 per cent, while the same dose of promethazine increased it 350 per cent. This corresponds to a relative activity of *0.28*. This same author also reported on the ether-anaesthesia prolonging action of the drugs. At the dose levels used, chlorpromazine elicited a 10.6 fold increase in the sleeping time, promethazine a 5.7 fold one; thus, the relative potency is *0.53*. According to LENKE, the ether-anaesthesia prolonging activity of promethazine is *0.20*, while the hexobarbital-anaesthesia prolonging potency is *0.14*. From the data of THEOBALD and DOMENJOZ one can calculate an AD_{300} value of 2 mg/kg for chlorpromazine and 20 mg/kg for promethazine. The relative potency of promethazine is, thus, *0.10*. In the experiments of KOPERA and ARMITAGE, a 300 per cent increase in the sleeping time was elicited by 6.3 mg/kg chlorpromazine. Promethazine was investigated at one dose level only: supposing a parallel run of the dose-effect curves, the AD_{300} value would be 20.5 mg/kg for promethazine, corresponding to an activity of *0.31*. In the experiments of SANDBERG, hexobarbital sleeping time in the mouse was increased by 102 per cent after 10 mg/kg chlorpromazine, while the same dose of promethazine produced an

Table 7. *Relative potencies of the compounds as described in the literature* (Chlorpromazine serves as standard with the reference-value = 1.)

Compound	Type of action						
	Sedative		Anaesth. prolonging		Block of cond. reflexes		Other
	Spont. activity	Antagonism against stimulants	Ether	Barbiturate	Primary	Secondary	
Promazine	0.30 (1) 0.22 (2) 0.24 (8)	0.22 (3)	0.69 (5)	0.44 (5)	0.39 (4) 0.14 (6) 0.61 (5)	0.11 (6)	0.50 (7) 0.32 (8) 0.40 (8) 0.50 (9)
Propionyl-promazine				1.0 (10)	0.66 (10)		1.6 (10)
Mepazine	0.16 (11) 0.17 (8) 0.16 (12)		0.29 (11) 0.41 (13) 0.13 (12)	0.17 (14)			0.11 (15)
RP 3300			0.22 (5)	0.12 (5)			
Pro-methazine	0.05 (4) 0.20 (1) 0.04 (2)	0.13 to 0.20 (16) 0.15 (3)	0.20 (19) 0.10 (17) 0.31 (18) 0.53 (5)	0.10 (21) 0.28 (5) 0.17 (13) 0.14 (19)	0.10 (20) 0.20 (4)		
Perphe-nazine	8.56 (22) 5.52 (2) 4.7 (24) 9.5 (25)			0.72 (25) 1.28 (25) 3.77 (25) 1.06 (25)	14.0 (22)		
Azacyclo-nol	0.05 (29)			0.05 (27)	0.08 (6)	0.07 (6)	
Meproba-mate	0.01 (28)	0.01 to		< 0.12 (31)	0.01 (28)	0.05 (6)	0.03 (30)
	0.03 (23) 0.01 (30) 0.02 (26) 0.02 (29) 0.02 (24) 0.06 (23)	0.02 (16) 0.01 (3)		0.04 (17) 0.05 (27)	0.07 (6)		0.03 (30) 0.07 (25)
Benacty-zine		0.28 (16)		0.24 (27)			
Hydroxy-zine	0.10 to 0.20 (5)	0.10 (16)		~ 0.12 (31)	0.15 (6)	0.07 (6)	

(1) Courvoisier et al., (2) Tedeschi et al. 1958, (3) Kouzmanoff et al., (4) Fellows and Cook, (5) Viaud, (6) Maffii, (7) Wirth, (8) Schlichtegroll, (9) Ayd, (10) Wirth et al., (11) Petersen et al., (12) Kopf and Nielsen, (13) Sandberg, (14) Nieschulz et al. 1958, (15) Plotnikoff and Green, (16) Thuillier and Nakajima, (17) Theobald and Domenjoz, (18) Kopera and Armitage, (19) Fenke, (20) Courvoisier, (21) Lessin and Parkes, (22) Irwin et al., (23) Kinnard and Carr, (24) Borsy et al., (25) Arrigoni-Martelli and Kramer, (26) Tedeschi et al, 1959, (27) Sacra et al., (28) Gatti, (29) Toekes, (30) Schallek et al., (31) Berger.

increase of 17.7 per cent. This corresponds to a relative potency of *0.17*. According to FELLOWS and COOK, 4.4 mg/kg chlorpromazine and 83 mg/kg promethazine were needed, resp., for a 50 per cent inhibition of the spontaneous motor activity of mice. Hence, the relative potency of promethazine is *0.05*. These authors investigated also the action on conditioned reflexes. 50 per cent block of the reflexes was produced by 2.4 mg/kg chlorpromazine and 12 mg/kg promethazine, a value corresponding to a relative potency of *0.20*. In the experiments of LESSIN and PARKES identical prolongation of anaesthasia was elicited by 3 mg/kg chlorpromazine and 30 mg/kg promethazine. This means a relative potency of *0.10*.

Summing up all these data, a mean relative activity in vivo of 0.19 can be calculated for promethazine. In our experiments we too, found 0.19, while the value calculated theoretically from the biochemical data is as well 0.19.

2. Promazine. According to COURVOISIER et al. the relative sedative potency of promazine is *0.30*. In the experiments of TEDESCHI et al. (1958), a 50 per cent inhibition of the spontaneous motor activity of mice was elicited by 4.7 mg/kg chlorpromazine and 21 mg/kg promazine. Hence, the activity of the latter is *0.22*. SCHLICHTEGROLL has investigated the sedative action of these drugs and has found that 5.3 mg/kg chlorpromazine and 25.5 mg/kg promazine, resp., were needed to produce 50 per cent sedation, a relative potency of *0.21*. In antagonizing the morphine-induced excitation of mice, the ED_{50} value in the experiments of KOUZMANOFF et al. was 1.4 mg/kg for chlorpromazine and 6.4 mg/kg for promazine, corresponding to a relative potency of *0.22*. According to VIAUD, 20 mg/kg promazine prolonged barbiturate sleeping time by 550 per cent, whereas the same dose of chlorpromazine increased it 1250 per cent. This would mean an activity of *0.44*. As to the ether-anaesthesia prolonging effect, this author reported a 1060 per cent prolongation after 20 mg/kg chlorpromazine and a 773 per cent increase after the same dose of promazine. Hence, the potency of promazine is *0.69*. Moreover, the effect of both drugs on the conditioned reflexes was investigated by VIAUD. 20 mg/kg chlorpromazine prolonged the normal reaction time from 2 to 28 sec, while promazine at identical dose level, to 18 sec. This would correspond to a relative activity of *0.61*. According MAFFII, the secondary conditioned reflex of the rat was inhibited 50 per cent by 1.75 mg/kg chlorpromazine and by 16 mg/kg promazine, a value corresponding to an activity of *0.11*. 50 per cent inhibition of the primary conditioned reflexes followed 11.6 mg/kg chlorpromazine and 84 mg/kg promazine. The potency of the latter drug is, thus, *0.14*. According to FELLOWS and COOK, a 50 per cent inhibition of the spontaneous activity of mice was brought about by 4.4 mg/kg chlorpromazine and 18 mg/kg promazine, values corresponding to an activity of *0.24*. These authors have also investigated the effect on conditioned reflexes. On this test, 50 per cent inhibition was produced by 2.4 mg/kg chlorpromazine and 6.2 mg/kg promazine. The potency of promazine is, thus, *0.39*. In the experiments of SCHLICHTEGROLL 2.8 mg/kg chlorpromazine and 8.9 mg/kg promazine were needed for 50 per cent inhibition of the postural reflexes in the mouse. This ratio gives an activity of *0.32*. To produce cataleptic effects, 4.35 mg/kg chlorpromazine and some 11 mg/kg promazine was necessary. From these data, one can calculate for promazine a relative activity of about *0.40*. According to AYD, promazine should be given at about twice as high a dose level as chlorpromazine, in order to ensure the same clinical effectiveness. This value corresponds to a potency of *0.5*. WIRTH compared the potency of chlorpromazine and promazine on various tests and found an average ratio of *0.50*.

As seen, the potency of promazine relative to chlorpromazine averages, in the experiments of various authors, is 0.35. In our own investigations,

we found a value of *0.49*, while the potency theoretically predicted from biochemical data is 0.55.

3. Meprobamate. According to GATTI, a sedative effect identical with that of 1 mg/kg chlorpromazine was produced by 100 mg/kg meprobamate. Thus the activity of this latter is, *0.01*. He investigated also the effect on conditioned reflexes and found that 0.1 mg/kg chlorpromazine and 10 mg/kg meprobamate produced reflex block, values corresponding to a relative activity of *0.01*. In the experiments of TEDESCHI et al. (1959), 50 per cent inhibition of the spontaneous motor activity of mice was brought about by 4.7 mg/kg chlorpromazine and 221 mg/kg meprobamate. Hence, the relative potency of meprobamate was *0.02*. SCHALLEK et al. compared the effects of these drugs on the spontaneous activity of mice, and found that 1.9 mg/kg chlorpromazine and 240 mg/kg meprobamate caused 50 per cent inhibition, a ratio of *0.01*. The same authors reported that 32 times as much meprobamate were required to produce identical EEG changes as well as equal fall in body temperature. From these data, one can calculate for meprobamate a relative activity of *0.03*. In the experiments of KOUZMANOFF et al. 50 per cent inhibition of morphine-excitation in the mouse was elicited by 1.4 mg/kg chlorpromazine and by 130 mg/kg meprobamate, a ratio of *0.01*. THUILLIER and NAKAJIMA compared these drugs on "souris tournantes": the mean effective dose of chlorpromazine was 2 mg/kg, while that of meprobamate lay between 150 und 200 mg/kg, corresponding to a relative activity of *0.01* to *0.02*. According to SACRA et al., 100 per cent prolongation of the phenobarbital sleeping time was produced by 2.2 mg/kg chlorpromazine and 42 mg/kg meprobamate. The potency of the latter is, thus, *0.05*. In the investigations of BERGER, 2.5 mg/kg chlorpromazine potentiated anaesthesia more than did 20 mg/kg meprobamate. The relative potency of meprobamate is, thus, less than *0.12*. THEOBALD and DOMENJOZ observed a 300 per cent increase in the sleeping time after 2 mg/kg chlorpromazine and 50 mg/kg meprobamate resp. The relative potency of the latter is, therefore, *0.04*. In the experiments of MAFFII, the secondary conditioned reflexes of the rat were inhibited 50 per cent by 1.75 mg/kg chlorpromazine and 32.5 mg/kg meprobamate, a ratio of *0.05*. 50 per cent inhibition of the primary conditioned reflexes was induced by 11.6 mg/kg chlorpromazine and 170 mg/kg meprobamate. The relative potency of meprobamate is, thus, *0.07*. PLOTNIKOFF and GREEN investigated the effect of these drugs on audiogenic seizures of the rat. A 50 per cent inhibition was brought about by 11 mg/kg chlorpromazine and 150 mg/kg meprobamate, values corresponding to an activity of *0.07*. According to the data of TOEKES, 50 per cent inhibition of the coordinated forced movement of mice ("rotarod" test) was elicited by 3 mg/kg chlorpromazine and 165 mg/kg meprobamate. The relative potency of meprobamate is, thus, *0.02*. Using the photocell-cage method, KINNARD and CARR found an ED_{50} value of 4.1 mg/kg for chlorpromazine and 144 mg/kg for meprobamate, a ratio of *0.03*. On the rotating rod test, 6.8 mg/kg chlorpromazine and 107 mg/kg meprobamate was needed to elicit 50 per cent reduction of the running time. This would correspond to a relative potency of *0.06*. According to BORSY et al., 50 per cent inhibition of the orientational hypermotility of mice was brought about by 1.4 mg/kg chlorpromazine and 92 mg/kg meprobamate, a ratio of *0.02*.

Summing up these data, we can conclude that the average relative potency of meprobamate is 0.03. It was 0.04 in our own experiments, while the potency calculated from the biochemical data is 0.02.

4. Hydroxyzine. GATTI found 1 mg/kg chlorpromazine to be equally effective as 5 to 10 mg/kg hydroxyzine. The relative potency of the latter is, thus, *0.10 to 0.20*. THUILLIER and NAKAJIMA compared these drugs on "souris tournantes" and found that 2 mg/kg chlorpromazine and 20 mg/kg hydroxyzine were equally

effective, a ratio *0.10*. In the experiments of BERGER, the anaesthesia-prolonging effect of 2.5 mg/kg chlorpromazine was elicited by about 20 mg/kg hydroxyzine, corresponding to a relative potency of about *0.12*. According to MAFFII, 50 per cent inhibition of the secondary conditioned reflexes was brought about by 1.75 mg/kg chlorpromazine and 24 mg/kg hydroxyzine. The activity of hydroxyzine is, thus, *0.07*. As to the primary conditioned reflexes, the ED_{50} value was 11.6 mg/kg for chlorpromazine and 76 mg/kg for hydroxyzine, which would correspond to a relative potency of *0.15*.

As seen, the data in the pertinent literature show for hydroxyzine a comparative activity in vivo of 0.12. We found a relative potency of 0.05, while that computed from biochemical data is 0.03.

5. RP 3300. Only a few data on this drug exist in the literature. According to VIAUD, RP 3300 produced 150 per cent and chlorpromazine 1250 per cent prolongation of barbiturate sleeping time, when given at 20 mg/kg dose level. This would correspond to an activity of *0.12*. In the same dose, chlorpromazine prolonged ether-anaesthesia by 1060 per cent, whereas RP 3300 increased it 233 per cent, a ratio showing a potency of *0.22*.

The mean value of these data is 0.17. This activity was also found in our experiments, while the biochemical data predicted a potency of 0.27.

6. Mepazine. According to PETERSEN et al., 50 per cent decrease in the spontaneous motor activity was produced by 2.4 mg/kg chlorpromazine and 15.5 mg/kg mepazine, a relative potency of *0.16*. Calculation from the data of these authors shows that 2.24 mg/kg chlorpromazine and 7.60 mg/kg mepazine, resp., caused a 300 per cent increase in the anaesthesia-time. Hence, the comparative activity of mepazine is *0.29*. SCHLICHTEGROLL compared the sedative action of these drugs. The ED_{50} value was 5.3 mg/kg for chlorpromazine and 31.5 mg/kg for mepazine, a ratio of *0.17*. In the experiments of KOPF and NIELSEN, 50 per cent inhibition of the spontaneous motor activity of mice was brought about by 2.4 mg/kg chlorpromazine and 15.5 mg/kg mepazine, a ratio of *0.16*. These authors also investigated the anaesthesia-prolonging action, finding that 1 mg/kg chlorpromazine and 8 mg/kg mepazine produced a 200 per cent increase in the sleeping time. From these data, one may calculate a relative potency of *0.13*. According to SANDBERG, 10 mg/kg chlorpromazine elicited an anaesthesia-prolongation of 103 per cent, while the same dose of mepazine 42 per cent, a relative activity of *0.41*. In the experiments of NIESCHULZ et al. (1958), 15 mg/kg chlorpromazine and 90 mg/kg mepazine were needed to block conditioned reflex responses by 50 per cent, a relative potency of about *0.17*. PLOTNIKOFF and GREEN found that 50 per cent protection against audiogenic seizures was elicited by 11 mg/kg chlorpromazine and 100 mg/kg mepazine, a ratio of *0.11*.

Sumarizing the data on mepazine, we see that an average relative potency of 0.19 was found in the experiments of various authors. In our investigations in vivo, we found a comparative activity of 0.21, whereas the relative potency predicted from biochemical data was 0.18.

7. Azacyclonol. According to SACRA et al., 100 per cent anaesthesia prolongation was elicited by 2.2 mg/kg chlorpromazine and 48 mg/kg azacyclonol, a relative potency of *0.05*. In the experiments of MAFFII, 50 per cent block of the primary conditioned reflexes was brought about by 11.6 mg/kg chlorpromazine and by 155 mg/kg azacyclonol, a comparative activity of *0.08*. The ED_{50} value in blocking secondary conditioned reflexes was 1.75 mg/kg for chlorpromazine and 25 mg/kg

for azacyclonol. From these data, the relative potency of the latter is *0.07*. TOEKES, investigating the effect on spontaneous motor activity in the mouse, found that 3 mg/kg chlorpromazine and 64 mg/kg azacyclonol, resp., caused 50 per cent inhibition, a ratio of *0.05*.

As seen, the data of the literature show an average relative potency of 0,06 for azacyclonol. We measured a value of 0.08, while the biochemical data predicted a relative potency of 0.05.

8. Propionylpromazine. This drug was, in the experiments of WIRTH et al., more active than chlorpromazine on certain tests, but less active on others. The average relative potency seems to lie very near to *1.0*. In our studies *in vivo*, a comparative potency of *0.71* was found, while the relative activity calculated from the data *in vitro* is *0.44*.

9. Benactyzine. In the experiments of SACRA et al., 100 per cent prolongation of anaesthesia was produced by 2.2 mg/kg chlorpromazine and 9 mg/kg benactyzine. Hence, the activity of the latter is *0.24*. The ED_{50} value on "souris tournantes" was 2 mg/kg for chlorpromazine and 7 mg/kg for benactyzine (THUILLIER and NAKAJIMA), a ratio of *0.28*.

These few data show for benactyzine an average relative potency of 0.26. We measured a comparative activity of 0,16, while the biochemical data predicted 0.27.

10. Perphenazine. According to IRWIN et al., 1.2 mg/kg chlorpromazine and 0.14 mg/kg perphenazine were necessary to produce a 50 per cent inhibition of spontaneous motor activity of rats, a relative potency of *8.56*. As to the inhibition of conditioned reflexes, these same authors found perphenazine *14* times more potent than chlorpromazine. From the data of ARRIGONI-MARTELLI and KRAMER, one can calculate that a 50 per cent inhibition of the spontaneous motor activity was brought about by 2.28 mg/kg chlorpromazine and 0.24 mg/kg perphenazine. The relative potency of the latter is, thus, *9.5*. These authors also investigated the hexobarbital-anaesthesia prolonging effect and found that 4 mg/kg chlorpromazine produced 106 per cent increase, while the same dose of perphenazine caused an increase of 76 per cent. This means a relative potency of about *0.72*. A 300 per cent increase in the pentobarbital sleeping time in the mouse was produced by 7.78 mg/kg chlorpromazine and 2.05 mg/kg perphenazine (assuming parallelism of the dose-effect curves). These data yield a relative potency of *3.77*. In the rat, the corresponding ratio for prolongation of hexobarbital anaesthesia was *1.28*, and for pentothal anaesthesia, *1.06*. In the experiments of TEDESCHI et al. (1858), 50 per cent decrease in the spontaneous motor activity of mice was elicited by 4.7 mg/kg chlorpromazine and 0.85 mg/kg perphenazine, a relative activity of *5.52*. The effect of both drugs on the orientational hypermotility of mice was investigated by BORSY et al. The ED_{50} values proved to be 1.4 mg/kg for chlorpromazine and 0.3 mg/kg for perphenazine. This corresponds to a relative potency of *4.7*.

On the average, all these data show a relative potency of 5.46 for perphenazine. The value calculated from the biochemical data is 5.63.

The relative potencies of the drugs as found by various authors, are summarized in Table 7. As seen, *the values theoretically calculated from biochemical data are practically identical with the activities found in the in vivo experiments of other authors.*

IV.

As seen above, the relative potencies calculated from biochemical (i.e. from *in vitro*) data correspond well to the *in vivo* potencies described in the literature. This comparison can be made in another way. An attempt should be made to calculate from the biochemical data of other investigators the potency to be expected *in vivo*. In addition, a comparison will be given between our own biochemical data and those found in the literature.

The inhibitory action of chlorpromazine on OP was confirmed by a great number of experiments (ABOOD; ABOOD and ROMANCHEK; ANDREJEW and ROSENBERG 1956a; CENTURY and HORWITT; ANDREJEW et al.

Table 8. *Inhibition by chlorpromazine of the oxidative phosphorylation and adenosine triphosphatase activity as described in the literature*

Oxidative phosphorylation			ATPase activity		
Concentration M	Inhibition in per cent	Reference	Concentration M	Inhibition in per cent	Reference
$2 \cdot 10^{-4}$	57.8	ANDREJEW and ROSENBERG 1956a	$2.82 \cdot 10^{-4}$	25.0	BERNSOHN et al. 1956a
$2.4 \cdot 10^{-4}$	50.0	YAMAMOTO et al.	$1 \cdot 10^{-3}$	56.0	ANDREJEW et al. 1956c
$2.98 \cdot 10^{-4}$	50.0	MAGEE et al.	$2.11 \cdot 10^{-4}$	25.0	Present study
$5 \cdot 10^{-5}$	~50.0	ABOOD			
$1.59 \cdot 10^{-4}$	50.0	Present study			

1956a; BERNSOHN et al. 1956a; MAGEE et al.). This holds also for inhibition of ATPase activity (ABOOD; ABOOD and ROMANCHEK; CENTURY and HORWITT; BERNSOHN et al. 1956a; BERNSOHN et al. 1956b; ANDREJEW et al. 1956b; ANDREJEW et al. 1956c; ANDREJEW and ROSENBERG 1956b). Table 8 shows the comparison of the inhibitory effects found by various authors. As seen, these values are essentially indentical with those observed in our own experiments. For example, 2.10^{-4} M chlorpromazine produced a 57 per cent inhibition of the OP both in our hands and in the investigations of ANDREJEW and ROSENBERG (1956a). MAGEE et al. measured the incorporation of ^{32}P into the creatine phosphate and ATP of brain slices. The ID_{50} value calculated from the data given was $2.98 \cdot 10^{-4}$ M for chlorpromazine; i.e., about twice as high as in our experiments. This slight difference is easily explained by the differences in the methods used. As to the inhibition of ATPase, the ID_{25} value of chlorpromazine was $2.11 \cdot 10^{-4}$ M in our experiments, and $2.82 \cdot 10^{-4}$ M in the experiments of BERNSOHN et al. (1956a). Accordingly, our quantitative *in vitro* results with chlorpromazine (this drug

was used for comparison throughout our investigations) are in good accord with the data of other authors.

As the to *in vitro* comparison of chlorpromazine with other drugs, only a few data are available. According to BERNSOHN et al. (1956c), the inhibitory effect of promethazine was 0.38 (when chlorpromazine = 1,0), while we measured a value of 0.24. MAGEE et al. compared the effects of chlorpromazine and azacyclonol on incorporation of ^{32}P into high energy phosphate bonds. 50 per cent inhibition of the incorporation was produced by $2.98 \cdot 10^{-4}$ M chlorpromazine and $2.82 \cdot 10^{-3}$ M azacyclonol, resp. Hence, the relative potency of azacyclonol was 0.11, a value identical with that found in our own experiments.

Table 9. *Potency expected in vivo of promethazine and chlorpromazine sulphoxide as calculated from the data in vitro of* ABOOD *and* ROMANCHEK

Compound	ID_{50}		Relative activity *in vitro*		Activity to be expected *in vivo*
	Oxid. phosph.*	ATPase**	Oxid. phosph.	ATPase	
Chlorpromazine	$4.8 \cdot 10^{-5}$ M	$1.66 \cdot 10^{-3}$ M	1.0	1.0	1.0
Promethazine .	$3.06 \cdot 10^{-4}$ M	$2.11 \cdot 10^{-3}$ M	0.16	0.75	0.12
Chlorpromazine sulphoxide .	$1.7 \cdot 10^{-3}$ M	$5.75 \cdot 10^{-3}$ M	0.03	0.29	0.009

* Supposing a slope value of 2.22 (found in the present experiments).
** Supposing a slope value of 4.46 (found in the present experiments).

Also interesting are the data of ABOOD and ROMANCHEK. These authors investigated the uncoupling effect of some phenothiazine derivatives, togheter with the action on ATPase. Unfortunately, they used the drugs at one single concentration level and, thus, no ID_{50} value was given. But making use of the slope-values found in our experiments, the *in vivo* potencies to be expected with promethazine and chlorpromazine sulphoxide can be calculated. This computation is shown in Table 9. As seen, the calculation from the data of these investigators gives for promethazine a relative potency of *0.12*. For chlorpromazine sulphoxide, a value of *0.009* may be calculated. The *in vivo* activity of promethazine is *0.18* (Table 7), while, on the rat and mouse, chlorpromazine sulphoxide is practically inactive (MORAN and BUTLER; DAVIDSON et al.). These data would show that *the in vivo effectiveness of a compound can be calculated with sufficient accuracy not only from our own biochemical data, but also from those reported in the literature.*

It is, however, to be noted that ABOOD and ROMANCHEK have also found compounds, which inhibited both OP and ATPase and which were not tranquilizers. Thus, diparcol (N-β-diethylamino-ethyl-phenothiazine) on both tests had identical activity to promethazine in inhibiting both OP and ATPase, a finding indicative of a relative potency *in vivo* comparable to that of promethazine. It would be

interesting to know something about possible chlorpromazine-like action of diparcol *in laboratory animals*; no such data was, however, found in the literature at our disposal. Another compound effective on both biochemical tests in ABOOD's experiment was phenothiazine itself. The uncoupling effect of this drug was evident also in our hands (unpublished experiments); quantitatively, it was comparable to that of chlorpromazine. Phenothiazine failed, however, to inhibit ATPase, even at a concentration level of 2.10^{-3} M. It is all the more difficult to explain this discrepancy, since ABOOD and ROMANCHEK used phenothiazine base, which is completely insoluble in water, while, in our experiments, the water-soluble potassium sulphate salt was employed. In any case, these observations call attention to the possibility that there are perhaps chemicals, which may inhibit both oxidative phosphorylation and ATPase activity without exhibiting chlorpromazine-like action in animal experiments. Of more than 80 drugs so far investigated, no such compound has been found. The possibility of such a drug cannot, however, completely neglected, even though it seems us rather unprobable.

The above-demonstrated quantitative parallelism between the *in vivo* and *in vitro* effects of tranquilizing drugs led us to the final conclusion that *the biochemical basis of tranquilizing drug action may be connected to the simultaneous inhibitions of oxidative phosphorylation and adenosine triphosphatase activity of brain (and especially in the cells of the hypothalamus)*. This statement, which holds *only for directly-acting drugs* and not for those of reserpine or tetrabenazine type, is supported by the following facts:

1. All compounds so far investigated that posses chlorpromazine-like central nervous system action inhibit both enzyme reactions, irrespective of the fact, whether they are chemically phenotiazines, diphonylmethans, aminoketones (DECSI and NÁDOR; DECSI 1958b) or other (e.g. diphenazine, DECSI 1960) molecular types.

2. Compounds inhibiting OP alone or ATPase alone are devoid of chlorpromazine-like central nervous system action.

3. The *in vitro* enzyme inhibitory effects are most marked on that part of the brain (hypothalamus), which is to be regarded as one of the main sites of action of tranquilizing drugs.

4. Between the actions of the drugs *in vivo* and *in vitro*, there is a close quantitative parallelism, permitting the *in vivo* potency of a compound to be predicted with sufficient accuracy from biochemical (i.e. from *in vitro*) data alone.

5. The *in vivo* activities found by various authors agree with the activities expected on the basis of our theoretical calculations.

6. The enzyme-inhibitory effects measured by us—the strength of which gives the basis for calculation of the *in vivo* potency—are essentially identical with the results of other investigators.

Summary

The effect of 13 tranquilizing drugs on brain metabolism has been investigated. Characteristic of all compounds was the ability to inhibit both the oxidative phosphorylation and the adenosine triphosphatase

activity of brain cells. A close quantitative relationship was found between the effects *in vitro* and *in vivo*; the relative effectiveness *in vivo* of a tranquilizer was determined by the strength of the inhibitory actions exerted on oxidative phosphorylation *and* adenosine triphosphatase activity. This quantitative paralellism between actions *in vivo* and *in vitro* is corroborated by data in the literature. It was concluded that tranquilizing drug effects (the manifestations measurable in animal experiments) are correlated with the simultaneous inhibition of oxidative phosphorylation and ATPase activity of brain, and especially of hypothalamic cells: i.e. with depression of energy production and energy utilization.

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The Significance of Mydriasis Produced by Amphetamine Sulphate

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With 8 Figures in the Text

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Dilated pupils are found in man after overdose with a number of sympathomimetic drugs, including the phenylethylamine compounds such as r- and d-amphetamine, methyl amphetamine, methyl phenidate and phenmetrazine, and the phenylethanolamine or phenylethanolamine-like substances ephedrine and pipadrol.

In spite of the fact that a number of sympathomimetic amines have been used as mydriatics, it has been assumed that the mydriasis and other signs of sympathetic over-activity found in man as a consequence of overdose are explicable by the anxiety, fear or terror frequently experienced during intoxication with the drugs, a point of view recently reiterated for phenmetrazine by Evans (1959). The results presented below (a part of which were communicated briefly to the second meeting of the International Collegium of Neuro-Psychopharmacology at Basle, Switzerland 1960) constitute evidence that the mydriatic effect of this type of substance is due both to a central action and a peripheral effect on the sympathetic post-ganglionic nerve endings in the iris.

Methods

The cats were anaesthetised by induction with ethyl chloride and ether, anaesthesia being maintained in the majority of animals with intravenous chloralose (80 mg/kg). Section of the mid-brain (the *cerveau isolé* of Bremer 1935) was perfomed in two animals, one of which was kept under ether until the section was completed and the other which was anaesthetised with chloralose. The technique for the *cerveau isolé* utilizes an approach through a fronto-parietal craniotomy as described by Bradley and Elkes (1957). The brains of these animals were fixed in 10% formol saline at the end of the experiment; the extent of the brain lesion was ascertained and found to be complete in both animals. Three spinal cats, one animal with the brain and spinal cord destroyed, and four cat *encéphale isolé* preparations (Bremer 1936) were made using a mid-line approach to the spinal cord through the atlanto-occipital membrane. One of the *encéphale isolé* preparations was also anaesthetised

with intravenous chloralose, and the other three with ethyl chloride and ether until the operative procedures were complete. In these, the margins of any skin incisions were infiltrated locally with procaine hydrochloride. In some cats, the superior cervical and vagal nodose ganglia together with a portion of the post-ganglionic trunks were previously extirpated aseptically (under pentobarbitone sodium anaesthesia 40 mg/kg) and 10—14 days allowed to elapse so that the sympathetic post-ganglionic fibres to the iris might degenerate. The vagal nodose ganglion was excised because sympathetic fibres may run in the cervical vagus (JONES 1932). In other animals, the ciliary ganglion was removed aseptically by the technique described by SHEN and CANNON (1936) and 7 days allowed to pass for degeneration of the parasympathetic post-ganglionic fibres to the iris. Both adrenal glands and any evident adrenal accessory tissue (usually found in the angle between the lower surface of the left renal vein and the inferior vena cava) were removed in all the animals but the *encéphale isolé* preparations, and the animals kept on artificial ventilation from the start of the experiment. This was to preclude the possibility of pupil enlargement due to anoxia and also of any effect on pupil size as a consequence of variation in respiration due to stimulation of the respiratory centre by the sympathomimetic drugs. The head of the cat was fastened in a holder and usually the nictitating membranes were tied aside (except with the *encéphale isolé* cats where the mouth of the animal was placed loosely round a mouth-bar and the nictitating membranes were not fixed in position). The eyes were equally illuminated, and to prevent drying, the cornea was periodically irrigated with warm saline. Photographs of the pupils were taken at a constant close distance from each animal. Drugs were injected into the cannulated right femoral vein and infusions were made with a PALMER slow-infusion pump. The blood pressure was recorded from the left femoral artery on a kymograph using a mercury mannometer connected to a siliconed glass cannula filled with heparin-saline. This was to ensure that the pupillary changes were not the result of a low blood pressure associated with otherwise undetectable deterioration of the animal. *Drugs used:* These included d-amphetamine sulphate, l-adrenaline bitartrate, l-noradrenaline bitartrate, chloralose and pentobarbitone sodium. Adrenaline and noradrenaline doses are given as base, amphetamine as the salt.

Results

1. Evidence that the mydriasis is not due exclusively to a central action of amphetamine. The continuity of the supranuclear connexions to the third nerves and of the sympathetic fibres from the hypothalamus

to the upper thoracic region of the spinal cord where the pupillo-dilator fibres emerge with the first, second and to a less extent the third thoracic nerves, was interrupted at the level of the mid-brain in two cats, the planes of section being shown in Fig. 1. The anterior section passes above the third nerve nuclei so the pupils are constricted (Fig. 2a), but they dilate as a consequence of the intravenous injection of amphetamine (Fig. 2b). The posterior sectionof the mid-brain damaged the third nerve nuclei so there was bilateral pupil dilatation in the other animal (Fig. 3a). Further dilatation of the pupils together with retraction of the nictitating membranes and widening of the palpebral fissures followed the intravenous injection of amphetamine (Fig. 3b). In this animal, the amphetamine was given after the effect of the ether had worn off. The mid-brain section precluded an action of amphetamine on the cerebrum as causative factor in the production of the mydriasis; damage to the third nerve nuclei meant that the pupillary dilatation produced by amphetamine in this type of unanaesthetised animal could not be a consequence of the inhibition of parasympathetic pupil constrictor tone.

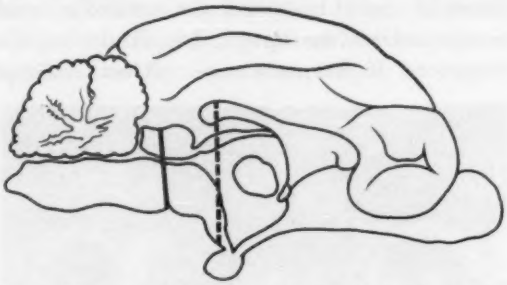

Fig. 1. Midline sagittal section of the cat's brain showing the planes of mid-brain section in two animals

Fig. 2a and b. Cat, 2.5 kg.; chloralose anaesthesia, with anterior mid-brain section. a Control size of pupils. b Further dilatation of the pupils 2 min. after the intravenous injection of d-amphetamine (2.5 mg/kg)

Bilateral and equal dilatation of the pupils after amphetamine was seen in an *encéphale isolé* preparation (section of the spinal cord at the

level of the first cervical vertebra). Destruction of the entire brain leaves the cat with pupils almost maximally dilated, but further increase in size of the pupil of a normally innervated (but not of a chronic sympathetic denervated) iris could be elicited by the intravenous injection of amphetamine. After destruction of the sympathetic centres in the spinal cord (by pithing) the mydriatic effect of amphetamine could still be obtained. This means that the mydriatic action of amphetamine in these animals was independent of any effect on the cerebrospinal axis. The dilatation of the pupils could not be ascribed to a release of adrenal hormones, as the adrenal glands had been removed.

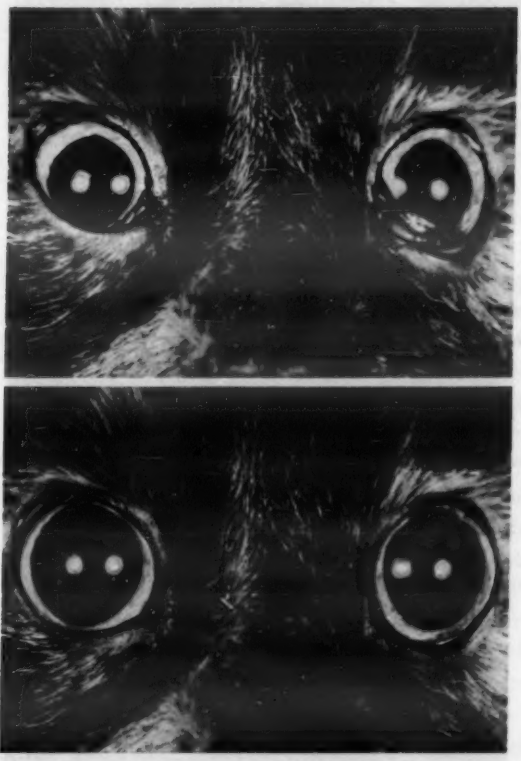

Fig. 3a and b. Cat, 4.0 kg. Posterior mid-brain section (cerveau isolé). a Control size of pupils. b Further dilatation of the pupils, retraction of the nictitating membranes and widening of the palpebral fissures 2 min. after the intravenous injection of 2.5 mg/kg d-amphetamine

2. Evidence that the mydriasis is due partly to a peripheral effect of amphetamine. These sympathomimetic amines dilate the pupil after acute resection of the ciliary ganglion or of the superior cervical and vagal nodose ganglia (MARLEY 1961). The site of action of these drugs must therefore be peripheral to the ganglia, either on receptors of the iris dilator muscle or at the sympathetic or parasympathetic post-ganglionic nerve endings in the iris. However, dilatation of the pupil with amphetamine still occurs after removal of the ciliary ganglion with a time lapse to ensure that the parasympathetic post-ganglionic nerve endings in the iris have degenerated (Fig. 4). On the other hand, after removal of the superior cervical and vagal nodose ganglia and time allowed for degeneration of the sympathetic post-ganglionic innervation to the iris, the intravenous infusion of amphetamine (Fig. 5b) or a

single intravenous dose of amphetamine (Fig. 5c) has scarcely any mydriatic action on the pupil of the denervated iris. In fact, there was some constriction of the pupil of the denervated iris after the single injection of amphetamine (Fig. 5c). That the pupil of the denervated iris is still capable of adequate dilatation was ascertained by injecting adrenaline intravenously (Fig. 5d) a substance which acts directly on the dilator pupillae muscle.

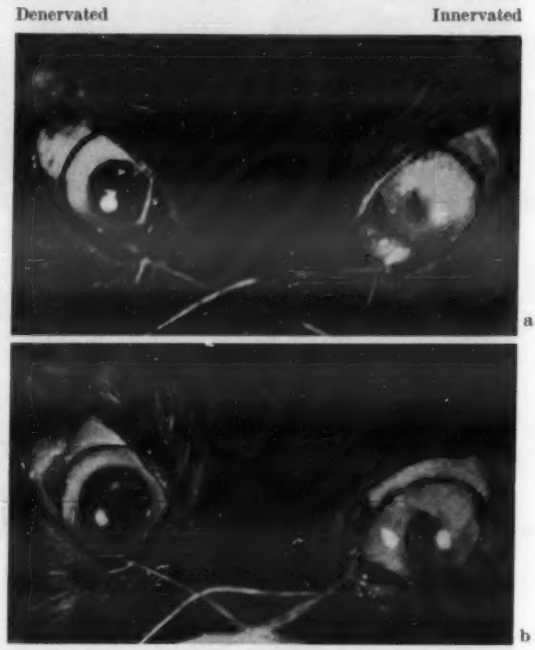

Fig. 4a and b. Cat 2.5 kg; chloralose anaesthesia. Right ciliary ganglion removed 7 days previously. a Control size of pupils. b Pupils 1 min after the injection of 2.5 mg/kg d-amphetamine

The possibility that the release of adrenal hormones by a direct action of amphetamine on the adrenal glands might contribute to its mydriatic effect was also studied. The average catechol amine content of each adrenal gland for male and female cats is 330.6 and 311.4 μg respectively (BUTTERWORTH and MANN 1957). A total of approximately three times the combined average amine content of the two glands was infused intravenously as a mixture of equal proportions of adrenaline and noradrenaline into adrenalectomized female animals. There was no enlargement of the pupil of normally innervated irides, and only half maximal dilatation of the pupil of chronic sympathetic denervated irides (Fig. 6) which are supersensitive to all the catechol amines. (The blood pressure which first was elevated with the infusion of the adrenaline and noradrenaline mixture, later returned to its resting level in spite of the continuation of the infusion). It is unlikely therefore that a sustained direct release of adrenal hormones contributes significantly to the mydriasis elicited by amphetamine with innervated irides.

The findings so far might suggest that the mydriatic action of amphetamine was of exclusively peripheral origin. However, in conscious cats with one innervated and one chronic sympathetic denervated iris, both pupils normally undergo rapid, frequent, and equal fluctuation

Fig. 5a—d.

in size. This change of pupil dimensions could hardly be due to intermittent spontaneous release of adrenal hormones (because of the brevity and equality of the pupil dilatation or constriction) for a denervated iris responds by greater and more sustained pupil dilatation than that of the innervated iris to adrenaline or noradrenaline. The pupil dilatation (at least for the denervated iris) must be due to inhibition of parasympathetic pupil constrictor tone, which is the mechanism usually of greater importance than alteration in sympathetic dilator tone for increase in pupil size (LANGWORTHY 1943). In such an animal, the intraperitoneal injection of 2 mg/kg of amphetamine produced either equal bilateral mydriasis, or dilatation of the pupil greater for the innervated than for the denervated iris. As amphetamine has just been shown to have scarcely any mydriatic action on the denervated iris in the animal anaesthetised with chloralose, the pupil dilatation produced must have been central in origin and the consequence of inhibition of pupil constrictor tone.

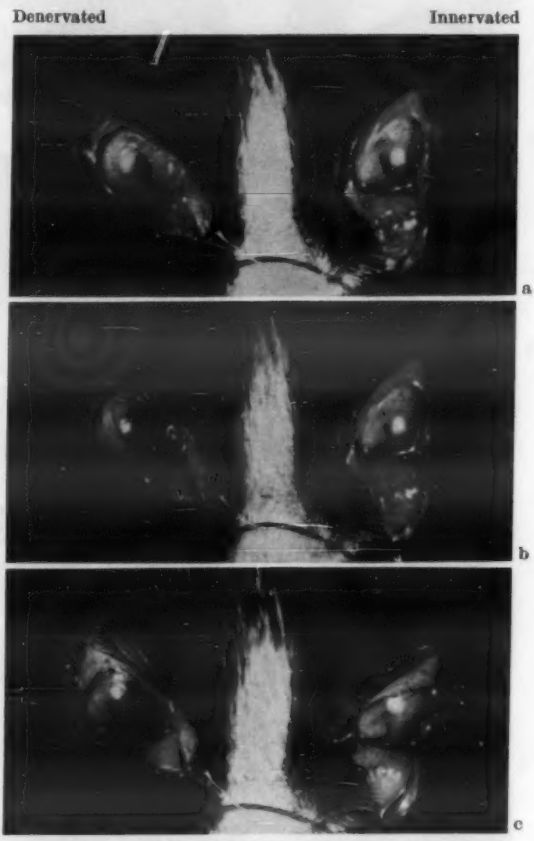

Fig. 6a—c. Cat 3.0 kg; chloralose anaesthesia and bilateral adrenalectomy. Right superior cervical and vagal nodose ganglia removed 14 days previously. a Control size of the pupils. b Pupils after the infusion of 200 μg adrenaline and noradrenaline (equal proportions) in 20 min. c After the infusion of 1800 μg adrenaline and noradrenaline in equal proportions for 80 min. (No alteration in size of the pupil of the innervated iris, but half-maximal dilatation of the pupil of the denervated iris)

Fig. 5a—d. Cat 2.5 kg., chloralose anaesthesia. Right superior cervical and vagal nodose ganglia removed 14 days previously. a Control size of the pupils. b 9.36 mg d-amphetamine has been infused intravenously over 255 min. There is minimal dilatation of the pupil of the denervated iris and some dilatation of the pupil of the innervated iris. c 1 min after the intravenous injection of 2.5 mg/kg d-amphetamine. Greater dilatation of the pupil of the innervated iris but constriction of the pupil of the denervated iris. d The pupil of the denervated iris dilated 1 min after the intravenous injection of 2 μg/kg l-adrenaline

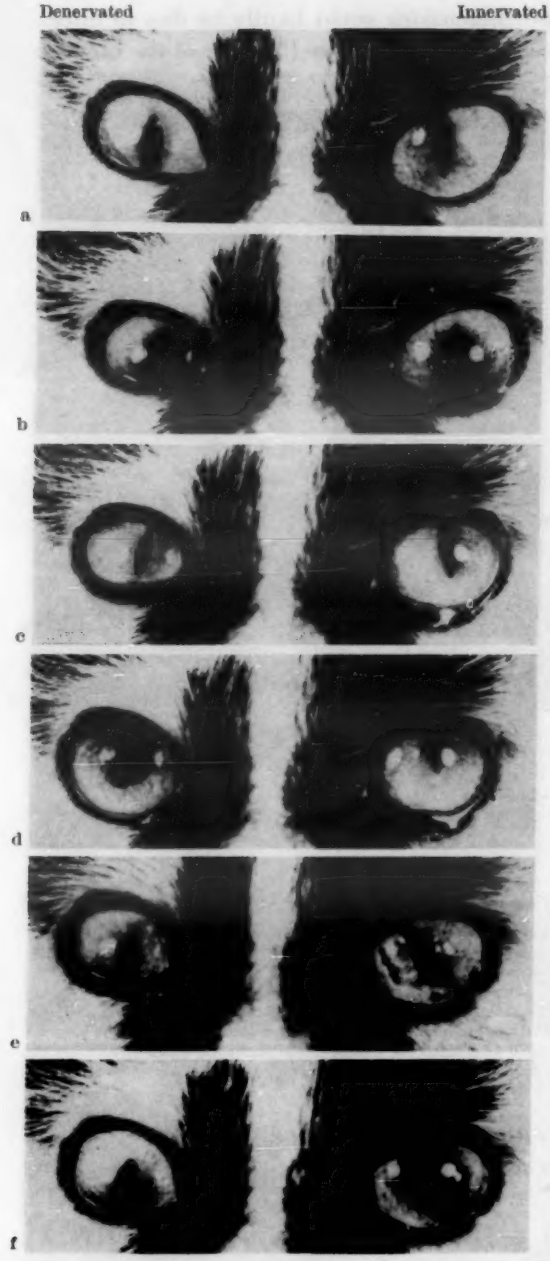

Fig. 7a—f. Cat 2.4 kg. *Encéphale isolé* preparation. Right superior cervical and vagal nodose ganglia removed 11 days previously. a Control size of pupils. b 12 mg of d-amphetamine infused intravenously over 20 min. Greater dilatation of the pupil of the denervated than of the innervated iris. c 2 min after the intravenous injection of 60 mg/kg chloralose; both pupils constrict. d 1 min after the intravenous injection of 4 μg/kg l-adrenaline. Half-maximal dilatation of the pupil of the denervated iris but only slight mydriatic action on the pupil of the innervated iris. e Control size of pupils. f A further 20 mg d-amphetamine infused intravenously over 85 min. Greater dilatation now of the pupil of the innervated than of the denervated iris

This was confirmed in animals in which section of the spinal cord precluded any relevant effect of amphetamine on peripheral structures, other than those innervated by the cranial nerves. Thus the intravenous infusion of amphetamine to cat *encéphale isolé* preparations, the animals having recovered from the ether and ethyl chloride anaesthesia, was accompanied by behavioural alerting (movements of the jaws, ears and vibrissae) and by either equal pupil dilatation of the innervated and denervated irides, or by greater dilatation of the pupil of the denervated than of the innervated iris (Fig. 7b), or *vice versa* (Fig. 8b). This paradoxical dilatation of the pupil of the denervated iris with amphet-

amine was unexpected. However, the intravenous injection of chloralose was followed by immediate and equal pupil constriction (Fig. 7c) and the disappearance of the behavioural alerting. Although chloralose has a depressant effect on smooth muscle (THOMPSON 1958) supersensitivity to adrenaline for the denervated iris was still found with this dose of chloralose (Fig. 7d). Chloralose itself does not favour pupil constriction as

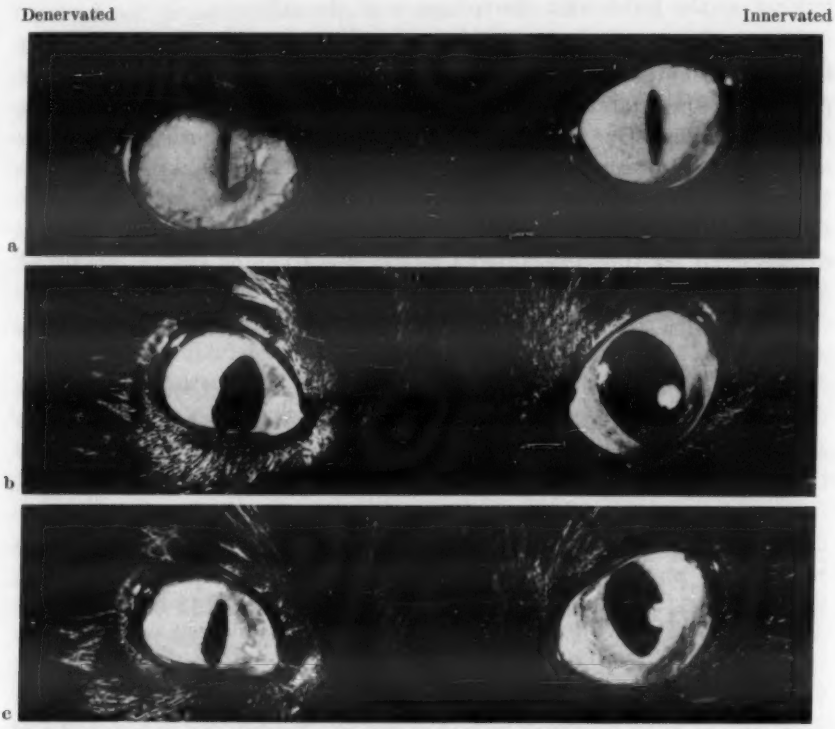

Fig. 8a—c. Cat 4.0 kg. *Encéphale isolé* preparation. Right superior cervical and vagal nodose ganglia removed 13 days previously. a Control size of pupils. b 36 mg d-amphetamine infused over 75 min. Greater dilatation of the pupil of the innervated than of the denervated iris. c 35 min after the slow intravenous injection of 15 mg/kg pentobarbitone sodium. Both pupils are smaller than in b, but the pupil of the denervated iris has constricted more than the pupil of the innervated iris

this was not elicited with the widely dilated pupils in spinal cats given intravenous chloralose in smaller or greater amounts (40 to 160 mg/kg) than its normal anaesthetic dose (80 mg/kg). The pupil contraction was unlikely to be due to a specific depressant effect of chloralose on the pupil dilator muscle, for the pupil size in the animal with intact pathways subserving the light reflex but anaesthetised with chloralose depends upon the amount of incident light reaching the retina, the pupil being constricted in bright illumination and dilating as this diminishes. The pupil dilatation was therefore presumably due to a

central action of amphetamine which ceased with the onset of anaesthesia. The further infusion of amphetamine to this animal (Fig. 7f) led to greater pupil dilatation of the innervated than of the denervated iris. That even here there might be a central as well as a peripheral action of amphetamine in spite of the chloralose, was suggested by the development of non-conjugate eye movements and myoclonic jerking of the head with the progress of the infusion.

In another cat *encéphale isolé* preparation, after the intravenous infusion of amphetamine (Fig. 8b) the central depressant substance pentobarbitone sodium was injected slowly until a dose of 15 mg/kg had been given. The behavioural arousal waned and both pupils gradually contracted during the subsequent 35 min, the pupil of the denervated iris approximating more to that of the control than did that of the innervated iris (Fig. 8c). This was again suggestive that the mydriasis even for the denervated iris was due to some central action of amphetamine.

The mydriasis produced by amphetamine is due therefore to both central and peripheral effects of the drug.

Reflex response to light. Impairment of the pupil reflex response to light was found after intravenous dosage with amphetamine in the animals anaesthetised with chloralose, the amount of drug required to do this exceeding that for the production of mydriasis. For example, in one experiment after the intravenous injection of 1.0 mg d-amphetamine, the transverse diameter of the pupil increased from 6 mm to 8 mm. The pupil contracted to 3 mm diameter on exposure to strong light. A total of 4.0 mg d-amphetamine was then given intravenously over the subsequent 50 min, the transverse diameter of the pupil increasing to 9 mm but on exposure to the same illumination as before, the pupil contracted only to 7.5 mm. Restoration of the full reflex response to light occurred after a lapse of 20 min. No impairment of the reflex response to light was found during the intravenous infusion of the large quantities of adrenaline and noradrenaline previously mentioned, nor for the dilated pupils immediately following removal of the adrenal glands with the associated release of large quantities of catechol amines.

Discussion

With intact conscious cats and with cat *encéphale isolé* preparations the pupils of both innervated and chronic sympathetic denervated irides were considerably dilated by amphetamine. It might seem that this mydriasis was exclusively of central origin and the consequence of inhibition of parasympathetic pupil constrictor tone. This conclusion was based on the abolition of or reduction of mydriasis in the *encéphale*

isolé cats after the administration of the central depressant substances chloralose or pentobarbitone sodium. Following the anaesthetic or in animals anaesthetised from the start of the experiment, amphetamine produced either none or minimal dilatation of the pupil of the denervated iris but would still dilate that of innervated irides.

However, the mydriatic action of amphetamine even in nonanaesthetised cat preparations was not exclusively of central origin for the drug produced dilatation of the pupils in spinal or pithed cats or in cat *cerveau isolé* preparations where a central action of the substance was precluded. It may be then that the mydriatic effect of small doses of amphetamine is of central origin. On the other hand, after the administration of or the ingestion of large quantities of amphetamine in conscious animals or persons, the pupil dilatation would be due to a combination of the central action of amphetamine together with its peripheral action at the sympathetic post-ganglionic nerve endings in the iris. This mechanism of action would apply also for other similar sympathomimetic amines such as phenmetrazine, and the results would be as equally valid for the human iris as for the cat's.

By virtue of their differential effect upon the innervated and the chronic sympathetic denervated iris in cats anaesthetised with chloralose, the sympathomimetic amines may be divided into three groups (MARLEY 1960a). These are (1) the phenylethylamines with no -OH groups on either the aromatic nucleus or the side-chain (for example amphetamine, phenmetrazine, methyl phenidate) the normal mydriatic action of which are absent or virtually absent on the denervated iris; (2) for substances with an -OH group either on the benzene ring, or on the β-carbon atom of the sidechain the mydriatic action remains after iris denervation but the threshold dose required to dilate the pupil of the denervated iris is raised (ephedrine and tyramine are most suitably included in this group) and (3) for substances with two -OH groups in the 3, 4 position on the benzene ring together with an —OH group on the β-carbon atom of the side-chain, the pupil of the denervated iris is supersensitive for threshold dose and for degree of and duration of mydriasis (all catechol amines). A point that has not been previously made and is of some relevance, is that this triple classification of structure-activity relations holds good only for the anaesthetised animal from which the adrenal glands have been removed. In the anaesthetised animal the mydriatic action of these amines is due either to an action on the post-ganglionic sympathetic nerve endings in the iris (as with amphetamine) or on muscle receptors in the dilator pupillae (as with adrenaline). Obviously, if the dose of amphetamine to an anaesthetised cat is increased sufficiently so that signs of central stimulation occur, the pupil dilatation produced will be a consequence of both central and peripheral effects of the drug.

It is possible that the assumption that the loss of mydriatic action of amphetamine on the chronic sympathetic denervated iris in the anaesthetised animal is due solely to degeneration of the post-ganglionic nerves to the iris is an over-simplification of the situation. After denervation, the pupil of the denervated iris is normally more constricted than that of the innervated iris presumably as a consequence of the unnopposed pupil constrictor tone in the affected iris. This alone would militate against pupil dilatation. Certainly after the intravenous infusion of carbachol which produces intense miosis by activation both of the post-ganglionic nerve endings to, and muscle receptors in the iris sphincter pupillae, the mydriatic effect of amphetamine is much reduced (MARLEY 1961), as it is also after intravenous reserpine which produces miosis of central origin mediated through the parasympathetic pathways (BEIN, GROSS, TRIPOD and MEIER 1953). Furthermore, it would seem that amphetamine may produce activation of the pupil constrictor muscle, an action that is not normally seen because of its powerful mydriatic effect (MARLEY 1961).

The release directly or indirectly of adrenal medullary hormones was shown not to be important for the mydriatic action of amphetamine on the innervated iris in anaesthetised animals, but was not excluded in either the *encéphale isolé* cats or the intact conscious cats. It was necessary here to simulate conditions as closely as possible to those in man. The adrenal glands were therefore left *in situ*. The fact that greater dilatation of the pupil of the denervated than of the innervated iris was sometimes found after amphetamine, was very suggestive that release of adrenaline and/or noradrenaline from some tissue site was occurring, and that the mydriasis was due both to a central action of amphetamine and to a direct action of adrenaline and/or noradrenaline on the sensitized dilator muscle of the denervated iris.

Even should release of adrenal catechol amines be contributory to the mydriasis produced by amphetamine, it must be remembered that dilated pupils may be found in man for up to 7 days after the withdrawal of amphetamine when large doses have been ingested. If indirect release of adrenal hormones had been occurring, this would imply prolonged excitation of the adrenal glands through their autonomic innervation. While there is abundant evidence in the cat for a continuous small output of catechol amines from the adrenal glands (VOGT 1952), when output of adrenal hormones is deliberately provoked, fatigue of the splanchnic-adrenal synapse soon supervenes even at low rates of excitation of the splanchnic nerve (MARLEY and PATON 1961). The duration of mydriasis following withdrawal of amphetamine is likely to be related to the time required for removal of and excretion of the amphetamine fixed to receptors in the brain and in the iris.

More critical appraisal of some of the findings in man after large doses of amphetamine might have cast doubt on the interpretation that the dilated pupils were a mere concomitant of the heightened mood change produced by the drug. For example, (1) dilated pupils may be found in persons suffering from amphetamine intoxication in whom the mood change is exclusively depressive, (2) after drug withdrawal, the anxiety, fear and terror, if previously present may wane, yet the dilated pupils persist, (3) marked anxiety may be present in the absence of mydriasis, and (4) there may be dilated pupils in persons with considerable abnormality of thought but little affective change. This is not to suggest that dilated pupils are not found in association with anxiety, fear or terror, but that there are alternative explanations for their presence with amphetamine intoxications. One method of distinguishing in man whether the mydriasis produced by amphetamine is primarily peripheral or central in origin would be to see whether it persisted or not after sedating the subject.

Impairment of the reflex pupil response to light was found in anaesthetised cats after the administration of amphetamine with a dose exceeding that for producing mydriasis. This was unlikely to be due to an action of amphetamine on the pathways subserving the light reflex, but merely a kinetic factor due to the tonic pupil dilatation produced by amphetamine which would limit pupil constriction from whatever cause. It may be that this impairment of the light reflex is only found when the peripheral action of amphetamine on the sympathetic post-ganglionic nerve endings in the iris predominates over its central effect. Lowenstein and Lowenfeld (1950) have remarked on the sluggish pupillary light reflex in animals after sympathomimetic drugs, and dilated pupils with diminished or absent reflex pupil changes to light and/or to convergence in man after sympathomimetic drugs were mentioned by Duke-Elder (1949). Connell (1958) in his superb monograph on amphetamine psychosis found that of his 42 cases of amphetamine intoxication, 12 had mydriasis and 3 an impaired pupillary light reflex. There are a number of physical signs common to intoxications with sympathomimetic amines in man (Bartholomew and Marley 1959; Marley 1960b), the signs including dilated pupils with impaired pupil reflex response to light and accomodation, tremor, enhanced limb muscle tone and tendon reflexes, and twitching of the facial musculature. It may be that a number of these features, apart from the dilated pupils, are due to a peripheral as well as to a central action of the drugs.

Finally, it was noted with the infusion experiments in the cats, that after the initial pressor effect of adrenaline and noradrenaline, the blood pressure returned to its original resting level in spite of the

continued intravenous infusion of these amines. However, there was no accomodation in terms of pupil size of the denervated iris to the infused catechol compounds. The mechanism of the rapid blood pressure accomodation is not known, for other tissues do not adapt so readily (Blacket, Pickering and Wilson 1950; Marley and Paton 1961).

Summary

The mydriatic action of amphetamine on innervated irides appears to be due to both central and peripheral effects of the drug.

Thus in intact conscious cats or cat *encéphale isolé* preparations, the intravenous administration of amphetamine produced considerable pupil dilatation of both innervated and denervated irides. This mydriasis could be abolished or much reduced, particularly for chronic sympathetic denervated irides, by the intravenous injection of central depressant substances such as chloralose or pentobarbitone sodium.

In non-anaesthetised spinal, pithed or *cerveau isolé* cats, the mydriatic action of amphetamine was elicited.

In anaesthetised cats, amphetamine dilated the pupil of innervated irides, but in doses unlikely to have any central action, amphetamine had little or no mydriatic action on the chronic sympathetic denervated irides. The mydriatic action of amphetamine could still be obtained after degeneration of the parasympathetic post-ganglionic nerve endings in the iris.

The release of catechol amines either directly or reflexly from the adrenal glands in anaesthetised cats is unlikely to contribute significantly to the dilatation of the pupils produced by amphetamine.

Impairment of the pupil reflex response to light may follow the intravenous injection of amphetamine, greater doses being required to do this than for the appearance of mydriasis.

The relation of these findings to the mydriatic action of some other sympathomimetic amines structurally related to amphetamine, and to the symptoms and signs found in man during intoxication with amphetamine was discussed.

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Effect of Amobarbital Sodium on the Flicker-Intensity Function for the Human Fovea*

By

G. W. Granger and H. Ikeda

With 4 Figures in the Text

(Received March 9, 1961)

Of the various psychophysiological techniques which have been used to quantify the effects of barbiturates on the central nervous system the flicker method is regarded as one of the most sensitive and reliable (Idestrom 1954). But although it has been observed that small doses of barbiturate depress the foveal critical flicker frequency (c.f.f.) (Roback *et al.* 1951; Landis and Zubin 1951; Aiba 1959) nothing is yet known about the psychophysical basis of this decrement. One possible explanation, which has already proved useful in accounting for the visual changes induced by other depressant agents (Granger 1959), is that barbiturates merely increase the luminance requirements for flicker recognition in the fovea as part of a quite general filter action on human visual thresholds. Idestrom (1954) has in fact observed that the luminance required to extinguish flicker at a fixed frequency of interruption of the light stimulus was higher following the administration of barbiturates than under normal conditions. But as his observations were limited to a single frequency (of 40 c/s) it is not known whether the filter effect would be constant over the range of frequencies encountered in foveal flicker. In view of the sigmoid form of the relation between the critical frequency (n) and log stimulus intensity (I) (the n-log I function as it is often called) the effect of a filter of a fixed density would be greater at the lower levels of retinal illumination than at the higher ones where the relation rapidly ceases to be linear as the c.f.f. approaches a maximum value[1].

If a simple filter hypothesis is valid the n-log I function should be of the same shape following the administration of a barbiturate as under normal physiological conditions but translated as a whole along the horizontal (log I) axis to higher intensities. On this view the value of the maximum c.f.f. should be unaffected by the drug for the two curves

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[1] Several investigators have found that at its upper end the curve bends over so that n-log I is not monotonic but the drop does not appear to be part of the essential phenomenon of flicker but due to the influence of extraneous variables.

would have the same asymptote. However, the possibility must also be considered that under the influence of barbiturate a normal maximum cannot be attained for the observed decrement in c.f.f. could be accounted for equally well in terms of a vertical shift of the n-log I curve along the frequency axis to lower critical frequencies. Either hypothesis is compatible with existing flicker data and with available pharmacological information concerning the effect of barbiturates on the central nervous system.

In order to decide between these alternatives it is necessary to cover a wide range of intensities: ideally, to cover the entire functional range of vision. For various practical reasons we have had to be content with less than this but we have nevertheless been able to cover a sufficiently wide range of illumination (about 20,000:1) to bring out the main features of the flicker-intensity function as previously established by HECHT and other workers (HECHT and VERRIJP 1933a). The measurements to be reported in the present paper were obtained with centrally-fixated test fields in white light falling within the fovea. In making them we have tried to eliminate as far as possible the effects of extraneous variables (e.g. fluctuations in pupil size) which obscure most of the previous results. We have also departed from previous practice by testing our hypotheses on individual rather than group data. The drug selected for study was amobarbital sodium, usually classified as an intermediate-acting barbiturate in terms of the duration of its effect (GOODMAN and GILMAN 1955). As the chief object of the experiment was to undertake further psychophysical analysis of the decrement in c.f.f. induced by single therapeutic doses of barbiturates we studied the effect of a single oral dose of 200 mg. This was of the same order as the doses of amobarbital sodium and amobarbital used by AIBA (1959) and IDESTRÖM (1954), respectively, in the investigations referred to above.

Method

Apparatus. The light source consisted of a tungsten-filament lamp underrun at a constantly-controlled voltage. An image of the filament (S) (see Fig. 1) was focused by a lens (L_1) upon a small aperture (A) which was in turn imaged by the photographic lens (L_2) in the plane of a rotating sector disc (Sh) with four 45° sectors removed to give a 1:1 light-dark ratio in each flicker cycle. As the beam was of very small cross-section at its point of interception by the sector disc it was possible to obtain an almost rectangular wave form. The diverging light was then collimated by a further lens (L_3), placed at focal distance from Sh, and finally brought to a focus by L_4 in the plane of the artificial pupil (P), which was 1mm. in diameter. Diaphragms (D) were inserted in the front focal plane of L_4 to limit the size of the test field to circular areas of

1°30′ and 45′ angular diameter as seen in Maxwellian view through P. A fixation point was provided in the form of a small black spot marked on a clear glass plate inserted at D. An additional red fixation point for use at the lowest luminance level of the test field was reflected into the eye from a small light source (not shown in the diagram) by a cover slip placed at 45° to the line of sight. Its luminance could be adjusted so as to be just comfortably visible by means of a rheostat.

The colour temperature of the source was approximately 2700° K, as determined by a colour temperature meter. The luminance levels of the test field were measured by a monocular matching method using a visual photometer and varied by means of calibrated neutral density

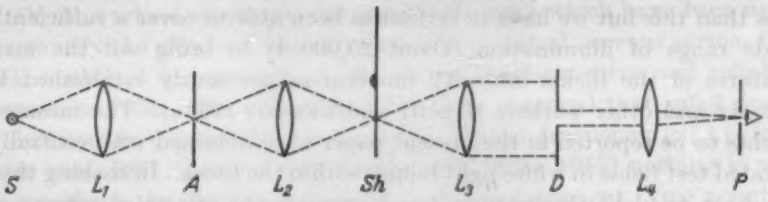

Fig. 1. Schematic diagram of apparatus. (For explanation of symbols see text)

filters. All stimulus intensities in this paper are expressed in terms of trolands[1] of retinal illumination. The sector disc was mounted on the shaft of a D.C. motor and its speed of rotation accurately controlled by a Ward-Leonard speed control unit. Frequency calibration, in c/s, was undertaken with the aid of a stroboscopic tachometer.

The apparatus was rigidly mounted and blackened housing and baffles used to reduce stray light to a minimum. The eyepiece containing the artificial pupil projected into a darkened cubicle in which the subject sat while making his observations. This was sealed against stray light at P.

Experimental procedure. The subject viewed the test field with his right eye placed immediately behind the artificial pupil (P), an adjustable chin rest and head supports being used to fix the position of the eye relative to the eyepiece. Each experimental session was preceded by a stay of 20—30 minutes in the dark. (Dark adaptation of the fovea, where the measurements were made, is complete in a much shorter time than this [HECHT 1921].) Control measurements were then made at five different intensity levels of the test field over a range of about four log units beginning with the lowest intensity. Before making a determination of the c.f.f. at a given intensity level the subject viewed the test field for 1.5 minutes with the frequency set above the fusion frequency so as to ensure adequate light adaptation of the retinal area under test.

[1] One troland is the retinal illumination produced when a surface of luminance 1 candela/sq. m. is viewed through an artificial pupil of area 1 sq. mm.

As a result of preliminary trials in which measurements were made starting from a frequency below as well as above the c.f.f., it was found desirable, in order to preserve the light-adapted state of the fovea and to maintain accurate fixation, to make determinations in one direction only, namely from fusion to flicker. As it is recognized that variations in certain features of n-log I curves (e.g. the slope and maximum c.f.f.) can occur as a result of a shift in the criterion used to judge "flicker" (HECHT and SMITH 1935) very careful instructions were given to the subject concerning the precise nature of the visual task. He was instructed to respond immediately he could detect any flicker in the test field by pressing a key which then signalled his response to the experimenter. It was emphasised that if for any reason (e.g. blinking, loss of fixation, blurring of the test field) he felt unable to make an accurate judgment of the onset of flicker he was to inform the experimenter who would then repeat the trial. The frequency was decreased in steps of about 0.5 c/s from a point just above the c.f.f. and the exposure time on which the subject based his judgment was always greater than one second.

At the beginning of our studies of the c.f.f. at least five and often ten determinations were made but, in accordance with the experience of HECHT and his associates, it was soon found that fewer readings were desirable to avoid eyestrain and fatigue. Moreover, estimates of the c.f.f. based on two or three settings agreed very closely with those based on ten settings. This was also the experience of HECHT *et al.* (1933) who determined thresholds from two settings in many instances.

The particular intensity range selected for study was limited mainly by difficulties experienced by our subjects in making judgments at very low and very high illuminations. At intensities lower than those recorded in our graphs all subjects had difficulty in maintaining central fixation and in deciding when flicker first appeared. At very high intensities they also experienced difficulty in making observations owing to interference from flicker in the surrounding field. This phenomenon is analogous to the well known effects of "glare" on other visual functions and may be attributed to entoptic scatter and dispersion in the ocular media (BARTLEY 1936). Although our subjects were instructed to concentrate on the test field and to disregard flicker in the surround the interference became so pronounced and the visual experience so unpleasant when the test field and surround were flickering at almost the same rate that it proved impossible to obtain an accurate estimate of the c.f.f. Even at the highest intensity for which measurements have been reported the observations were subject to considerable variation and the recorded values are based on at least five settings instead of the usual two or three. It should be mentioned that the disturbing effect of flicker in the surround can be overcome by using an illuminated surround (HECHT

et al. 1933) but this method has certain other disadvantages, as pointed out by CROZIER *et al.* (1937).

Using the above procedure data were obtained from each subject in two separate experimental sessions. On the first occasion control measurements were first obtained and the subject was then given 200 mg amobarbital sodium orally (in capsule form with about 200 ml of water) whilst he remained in darkness. During the following two and a half hours repeated determinations of the c.f.f. were made very rapidly every few minutes or so (allowing for rest pauses) so as to plot the time course of the drug effect. On the second occasion the procedure was the same except that additional measurements were made over a wider range of illumination during the period (between 70 and 90 minutes after the drug had been administered) when the drug was having a maximum and constant visual effect.

Subjects. Data were obtained from six male subjects, all of whom were free from visual defects and other conditions known to affect the c.f.f., and had some previous experience in making judgments of the foveal c.f.f. Measurements obtained on three previous occasions had established that their n-log I curves were highly reproducible and all of the same basic shape, as previously found by HECHT and many others. During a period of three hours the range of normal intra-individual variation for a given luminance level never exceeded 1 c/s.

Results and Discussion

Effects of the drug on the n-log I function are shown in Figures 2, 3 and 4; each figure shows the results for two subjects. The open circles denote the control data and the filled-in circles the barbiturate data obtained between about 70 and 90 minutes after the drug had been administered. The limits of normal intra-individual variation in the c.f.f. (1 c/s) observed over a period of three hours are defined by the (vertical) diameters of the open circles. The control curves display the typical features of n-log I data obtained with centrally-fixated fields falling within the fovea. Over an intensity range of about three log units there is a direct logarithmic relation between intensity (I) and the critical frequency (n) (the Ferry-Porter law: $n = a \log I + b$) but at the highest intensities the relation ceases to be linear as the c.f.f. approaches a maximum value at a retinal illumination of about 10,000 trolands. Although there is some individual variation[1] in this maximum critical

[1] The values of the maximum c.f.f. are very similar (about 39 and 39.5 c/s) for the two subjects who viewed the 1°30′ field and for three of the four subjects who viewed the 45′ field (35.5, 36 and 36.5 c/s) but for subject A the maximum was considerably higher (40 c/s). The tendency for the maximum c.f.f. to be higher for the larger field is to be expected on the basis of previous research (e.g. HECHT and SMITH 1935; LLOYD 1952).

frequency the basic form of the function is similar in all six subjects and conforms closely to that found by HECHT and many previous workers

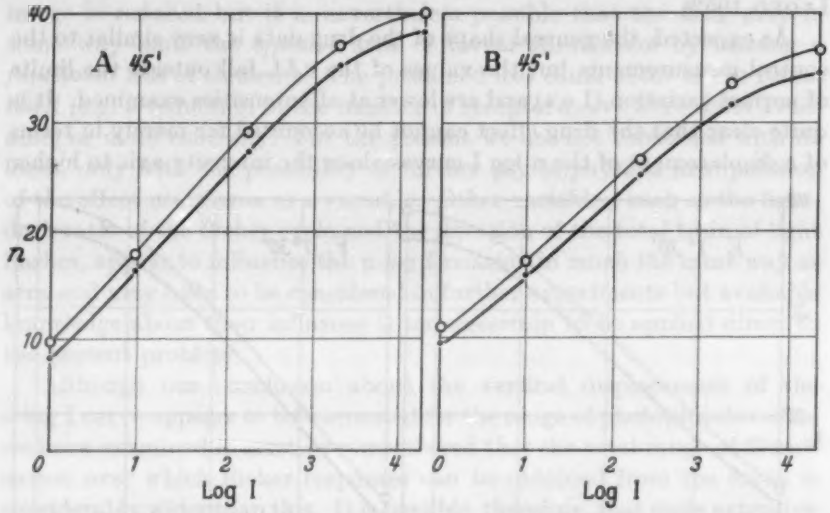

Fig. 2. Critical flicker frequency (n), in c/s, as a function of log intensity (I), in trolands, for a test field of 45′ angular subtense. ○ control data; ● barbiturate data. (Subjects A and B)

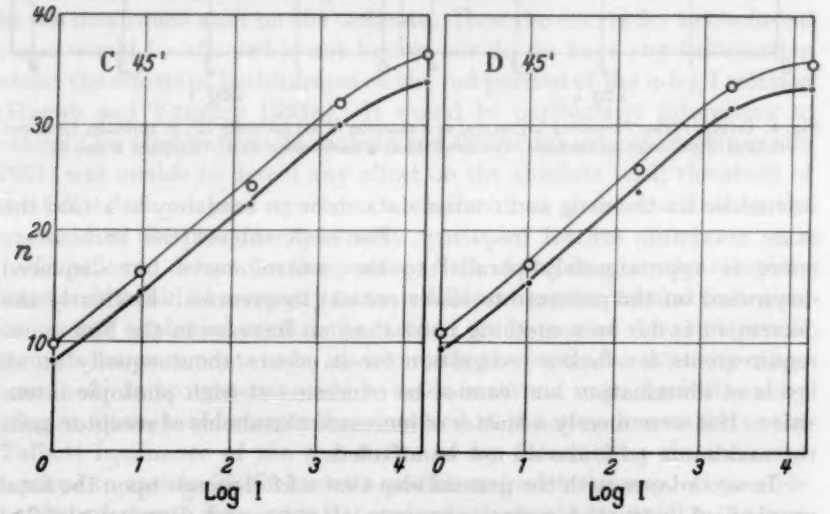

Fig. 3. Critical flicker frequency (n), in c/s, as a function of log intensity (I), in trolands, for a test field of 45′ angular subtense. ○ control data; ● barbiturate data. (Subjects C and D)

(HECHT and VERRIJP 1933a; HECHT *et al.* 1933; ROSS 1938; LLOYD 1952). Within the range of intensities between about 1 and 1000 trolands, where the data fall with fair precision on a straight line, the slope of the curves

is similar in value to that found by earlier investigators who have used centrally-fixated fields falling within the fovea (HECHT and SMITH 1935; LLOYD 1952).

As expected, the general shape of the drug data is very similar to the control measurements but the values of the c.f.f. fall outside the limits of normal variation (1 c/s) and are lower at all intensities examined. It is quite clear that the drug effect cannot be accounted for merely in terms of a displacement of the n-log I curves along the intensity axis to higher

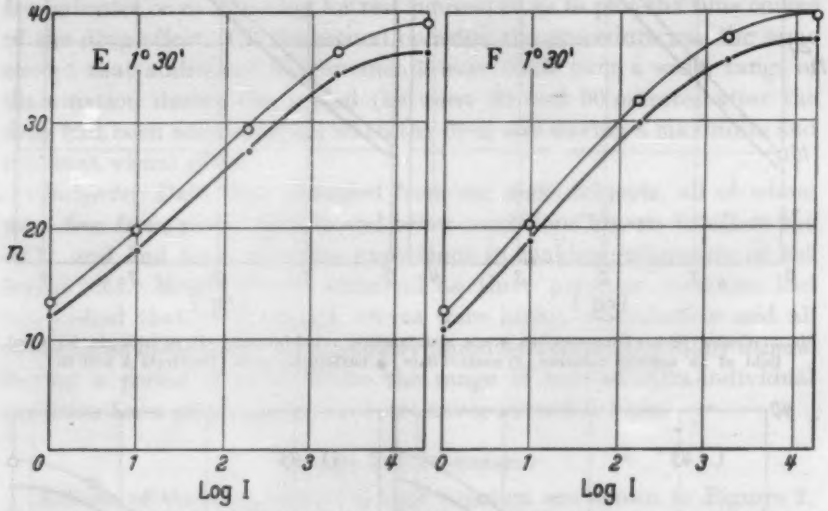

Fig. 4. Critical flicker frequency (n), in c/s, as a function of log intensity (I), in trolands, for a test field of 1°30′ angular subtense. ○ control data; ● barbiturate data. (Subjects E and F)

intensities for the drug and control data show no tendency to attain the same maximum critical frequency. For each subject the barbiturate curve is approximately parallel to the control curve but displaced downward on the ordinate to lower critical frequencies. Evidently the decrement is due to something more than an increase in the luminance requirements for flicker recognition for it occurs about equally at all levels of illumination and cannot be overcome at high photopic intensities. If it were merely a matter of increased thresholds of receptor units the maximum c.f.f. should not be affected.

In accordance with the general idea that c.f.f. depends upon the total number of activated neural elements (HECHT and VERRIJP 1933b; CROZIER and HOLWAY 1939) the impairment of critical frequency over the entire range of illumination suggests a loss of elements. The downward displacement of the entire n-log I curve is in fact analogous to reducing the size of a centrally-fixated (foveal) field (HECHT and SMITH 1935; LLOYD 1952). Thus, under the influence of the drug it is as if the

fovea were being stimulated by a stimulus of smaller areal subtense. There is, of course, no reason to suppose that the actual size of the retinal image is reduced but it is nevertheless possible that the drug may in some way limit the *effective* area of foveal stimulation by causing a *functional* loss of elements. The presumed loss could occur in the retina itself (e.g. a reduction in the number of receptor-nerve fibre units available) or more centrally. For the present we are not concerned with its locus, only with the possibility of further psychophysical manipulation of the effect using area as a variable. Other variables, such as the light-dark ratio of the flicker cycle and the duration of the total train of light flashes, appear to influence the n-log I relation in much the same way as area and may have to be considered in further experiments but available knowledge about their influence is too uncertain to be applied direct to the present problem.

Although our conclusion about the vertical displacement of the n-log I curve appears to be warranted for the range of photopic intensities we have examined it must be remembered that the total range of illumination over which flicker responses can be obtained from the fovea is considerably wider than this. It is possible, therefore, that more extensive observations at very high and very low illuminations might reveal a slight shift of the foveal n-log I function to higher intensities in addition to the downward shift on the ordinate. How the curves for extra-foveal cones would be affected is not known nor do we have any information about the effects of barbiturates on the rod portion of the n-log I relation (HECHT and VERRIJP 1933a). It would be particularly interesting to extend the observations to include rod flicker for one of us (GRANGER 1961) was unable to detect any effect on the absolute (rod) threshold of the fully dark-adapted eye from a similar dose of amobarbital to the one used in the present experiment.

In order to determine the precise effect of the drug on the complete function it would be necessary to use a different psychophysical technique for, as was explained earlier, the method we have used does not permit accurate measurements to be made outside the particular intensity range examined. Improvements could be made in several ways, for instance by using an illuminated surround maintained at the same luminance as the (Talbot) luminance of the test field and by determining the intensity required to extinguish flicker at a predetermined frequency. If possible the steps between successive determinations should be smaller (5 c/s or less in terms of frequency) and each experimental point should be determined systematically from a "frequency-of-fusion" curve with randomised stimulus presentation and controlled exposure of the train of light flashes. The latter technique could also be used very satisfactorily with the test field superimposed on a steady background illumination, as in

increment threshold measurement. Making determinations in the way suggested would, however, be a very time-consuming procedure and it would be necessary to prolong the duration of the drug effect in a given session and combine measurements from different sessions in order to secure adequate data.

Summary

Measurements of the foveal critical flicker frequency (c.f.f.) were obtained from six subjects over a wide range of retinal illumination (I) before and after the oral administration of 200 mg amobarbital sodium. Although the drug did not affect the basic shape of the sigmoid function relating c.f.f. to log I the curves of all subjects were displaced downward on the frequency axis to lower critical frequencies. As this effect is analogous to reducing the area of foveal stimulation it was suggested that the drug may cause a functional loss of neural elements either in the retina itself or more centrally. Possible improvements in technique to enable observations to be extended to higher and lower intensities and to other parts of the retina were briefly discussed.

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From The University of Oklahoma College of Pharmacy

Mediated Acquisition of a Fear-Motivated Response and Inhibitory Effects of Chlorpromazine*

By

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With 1 Figure in the Text

(Received November 14, 1960)

Extensive clinical experience with chlorpromazine and its congeners testifies to the ability of such agents to reduce agitation and manifestations of anxiety in human subjects. Although greatest emphasis has been placed on its effect on psychotic behavior, Wikler finds that "in the treatment of psychoneuroses, there appears to be general agreement that chlorpromazine is useful as a palliative and as an aid to psychotherapy." Relief of symptoms and enhancement of psychotherapy during chlorpromazine treatment of neuroses are both generally attributed to some inhibitory effect of the drug on emotional excitability.

Animal experimentation has yielded evidence for seemingly analogous effects of chlorpromazine on the emotional state of infrahuman subjects. The most common demonstration of an inhibition by chlorpromazine of fear- or anxiety-associated behavior has been the conditioned avoidance response (CAR). Inhibition of the acquisition of an avoidance response in rats was shown by Lynch *et al.* Selective inhibition of performance of the fear-motivated CAR over the pain-motivated escape response has been demonstrated repeatedly in rats by Cook and Weidley, Courvoisier, McMurray and Jaques, R. E. Miller *et al.*, (1957), and others. The CAR test has been widely used for the characterization of potential new "psychoactive agents" in spite of differences among psychologists in their interpretation of the events involved in avoidance conditioning and in spite of uncertainty of the mechanism of drug effects on the CAR.

Interpretation of the basis for learning and performance of the CAR, as stated by McMurray and Jaques, and others, is usually based on the stimulus-response concept of learned behavior and on the drive-reduction hypothesis of reinforcement as propounded by N. E. Miller. This interpretation is presented diagrammatically in Fig. 1a and may be summarized with pharmacological emphasis as follows: (1) an escape response to a shock stimulus is learned; (2) concurrently, unconditioned fear responses evoked by shock become conditioned to an exteroceptive

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auditory or visual signal which is presented contiguously with the shock; (3) the conditioned fear responses (or "conditioned fear") to the signal supply interoceptive "fear stimuli" which are able to elicit the conditioned response in the absence of the shock; (4) the conditioned fear thus serves as an acquired drive and responses to the "fear stimuli" such as hurdle-crossing are reinforced as they cause reduction of this fear drive; (5) pharmacological reduction of the intensity of conditioned fear, or pharmacological interference with the functioning of "fear stimuli" would result in a reduction of the CAR. Thus, inhibition or suppression

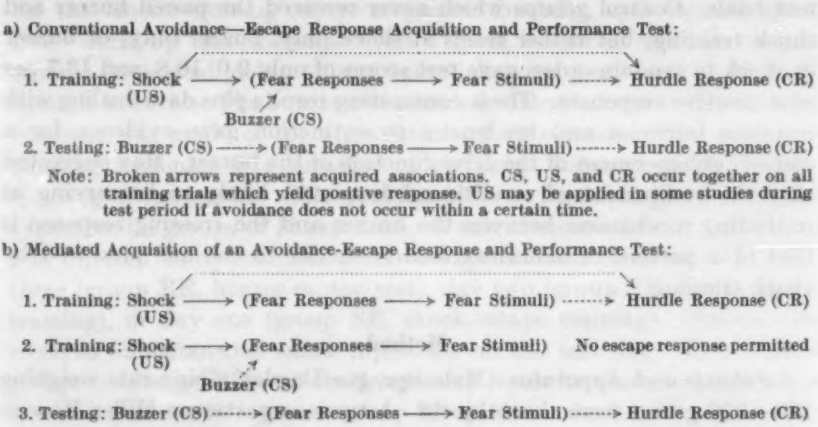
a) Conventional Avoidance—Escape Response Acquisition and Performance Test:
1. Training: Shock (US) → (Fear Responses → Fear Stimuli) → Hurdle Response (CR)
Buzzer (CS)
2. Testing: Buzzer (CS) → (Fear Responses → Fear Stimuli) → Hurdle Response (CR)
Note: Broken arrows represent acquired associations. CS, US, and CR occur together on all training trials which yield positive response. US may be applied in some studies during test period if avoidance does not occur within a certain time.
b) Mediated Acquisition of an Avoidance-Escape Response and Performance Test:
1. Training: Shock (US) → (Fear Responses → Fear Stimuli) → Hurdle Response (CR)
2. Training: Shock (US) → (Fear Responses → Fear Stimuli) No escape response permitted
Buzzer (CS)
3. Testing: Buzzer (CS) → (Fear Responses → Fear Stimuli) → Hurdle Response (CR)
Note: CS and CR never occur together before test period. US is not applied during test trials

Fig. 1 a and b. Comparison of experimental designs for psychopharmacological observations

of the CAR as observed with chlorpromazine is usually attributed to such fear reduction or alteration of "fear stimuli".

It seems that additional experimental designs to promote further characterization of the action of chlorpromazine on fear-motivated behavior would be valuable. An experiment which contributes significantly to the psychopharmacological interpretation of the clinical and experimental effects of chlorpromazine is presented here. The design follows a study by MAY which demonstrated that fear may serve not only as an acquired drive, but also as mediator for the transfer of a conditioned response to a new eliciting stimulus, a process referred to as *secondary* stimulus generalization. That is, the generalization is based not upon the similarity of the external stimuli as in primary stimulus generalization, but upon the similarity of internal stimuli evoked by two sets of conditions. Fig. 1 b presents this experimental procedure in diagrammatic fashion in comparison with the CAR procedure. Although superficially resembling the CAR it may serve as a useful experimental model because of the difference in the training process from that

for the CAR. While providing a more complex learning situation for the subjects, it also somewhat facilitates study of drug effects on conditioning of a fear-motivated response.

May first established an escape response to shock in rats under conditions tending to prevent responses conditioned to environmental cues. Secondly, a buzzer was paired with shock under conditions preventing the performance of any consistent escape response. The rats were then tested for escape responses to the buzzer without shock. Groups receiving such treatment gave 70 and 84.5 per cent positive responses on test trials. Control groups which never received the paired buzzer and shock training, but rather received shock only, buzzer only, or buzzer or shock in random order, gave test scores of only 9.0, 10.8, and 13.7 per cent positive responses. These contrasting results plus data dealing with response latencies and tendencies to extinction gave evidence for a distinct enhancement of the drive function of the buzzer. May suggested that the component of conditioned fear most likely to be serving as mediating mechanism between the buzzer and the crossing response is that of a pattern of neural excitation similar to the one produced by shock training.

Method

Subjects and Apparatus. Male Sprague-Dawley albino rats weighing 230—330 grams were the subjects. A two-compartment Miller-Mowrer shuttle box with separately electrified grid floors and a center barrier-partition with roller top was used as by MAY. Shock wasdelivered through the grid of either compartment with a 2.5 megohm resistor in series, using a measured grid voltage of 100 v. (reduced to 75 v. in one portion as described below). A removable inverted U-shaped partition was used in phase two of training to convert part of one side of the box into a non-escape "shock pen" (differing in size and appearance from the compartments of the shuttle box) which restricted the rats to very minimal movement and provided for no escape from shock.

Procedure. There were three phases each of which occupied one of three consecutive experimental days. The first phase consisted of trials training the rats to make a barrier-crossing escape response. The buzzer used in the latter two phases was not sounded. Without warning each rat was shocked until a barrier crossing was made. The shock intensity was such that this occurred rather soon after a burst of vigorous activity. Thirty shock-escape trials were given in units of ten. Subjects were rested 45 minutes between units and 20 to 40 seconds between successive trials. The purpose of this phase was to condition the internal fear responses and fear stimuli associated with shock and pain to the barrier-crossing response.

Phase two consisted of trials in which a rat was confined in the shock pen. The subject was exposed to the sound of a buzzer for five seconds prior to the onset of shock and also during the shock. Fifteen consecutive non-escape shock-buzzer trials were given at 20 second intervals. The duration of shock was increased from 2 seconds to 3 seconds to 4 seconds in successive groups of five trials. This phase served to condition the shock-evoked, drive-producing fear responses and "fear stimuli" to the buzzer[1].

In phase three the subjects were placed in the shuttle box arranged for the barrier-crossing escape response. Initially, each rat was given five "warm-up" shock-escape trials without buzzer in rapid succession. After a 30 second delay thirty test trials were conducted at 30 to 60 seconds intervals. The buzzer was sounded without shock until a crossing response was made or for a maximum of 10 seconds. The number of crossing responses is a measure of the drive function of the buzzer.

Drug Experiments. Chlorpromazine[2] was tested in three different experimental groups of 10 rats each. A subcutaneous dose of 1.25 mg/kg was injected thirty minutes prior to experimental trials on either day three (group BE, buzzer-escape test), day two (group SB, shock-buzzer training), or day one (group SE, shock-escape training). Control rats received subcutaneous saline injections on the test day. Two control groups trained at different shock intensity levels became necessary when it was impossible to complete group SE at 100 v. shock level. Therefore, a new group SE and one control group were trained with 75 v. shock while groups SB and BE and the other control group were trained at 100 v. Group SB was completed at the higher voltage with substitution of two replacement subjects. The difficulty encountered was a tendency for rats receiving chlorpromazine on shock days (1 and 2) to bite the grid to a degree seldom seen by us in undrugged rats. Voltage was reduced because of the occurrence of damaging and disabling effects in the drugged rats. This may be in accord with results indicating a synergistic interaction of chlorpromazine and electroconvulsive shock (Brown *et al.* and Elam *et al.*). A new type of shock grid which would eliminate this difficulty has been suggested recently by Dinsmoor.

Results

Table 1 presents the mean and standard deviation of the number of crossing responses on day three for each experimental and control group and probability values for the comparisons between experimental and

[1] In a preliminary experiment, phases one and two were conducted concurrently on two days in alternating groups of shock-escape trials, buzzer-shock trials, and shock-escape trials, as in May's paper. This was altered to permit drug administration during either phase one or two, as well as during phase three.

[2] As Thorazine, generously supplied by Smith, Kline and French Laboratories.

Tabelle 1. *Results of thirty-trial test for performance of the mediated response (barrier-crossing to buzzer) with chlorpromazine treatment prior to or during the test*

Group *	Responses		Probability of Difference From Control
	Mean (S. D.)	% Control Responses	
Control (75 v.)	14.9 (10.8)	100	—
SE (Drug during Shock-Escape Trials) .	11.7 (6.9)	78.5	$p = 0.20$
Control (100 v.).	22.0 (6.2)	100	—
SB (Drug during Shock-Buzzer Trials) .	13.0 (9.1)	59.1	$p < 0.01$
BE (Drug during Buzzer-Escape Test) .	2.8 (3.1)	12.7	$p < 0.001$

* Ten rats per group.

corresponding control groups. The Kruskal-Wallis and Mann-Whitney tests (SIEGEL 1956) were used for statistical analysis. Response scores are also expressed as per cent of control response level. It can be seen that groups which received chlorpromazine treatment during phase two or three showed a highly significant reduction in number of barrier-crossings compared to the control group. The response levels of groups SB and BE also differed significantly from each other ($P < 0.02$). Group SE gave an average performance of 78.5% of its control group, but the difference was not statistically significant.

On training day one some response latencies for the last ten trials (100% responses) were recorded. To check the equivalence of groups in acquisition of the escape response, the groups trained at the same shock level were compared. There was no significant difference between mean latencies for the ten trials — group SE vs. Control (75 v.), 11.0 and 11.2 seconds; group SB vs. Control (100 v.), 7.8 and 7.0 seconds. The comparison of SE and Control suggests an absence of drug influence on escape response acquisition.

In a pilot study (referred to in footnote under Methods) 1.25 mg/kg chlorpromazine was administered subcutaneously two hours rather than one-half hour prior to day three testing. Inhibition of crossing responses was of the same degree as in the main experiment. Mean number of responses for 8 drugged rats was 6.1 while a control group of equal size averaged 21.2 ($P < 0.01$). Analysis of response latencies of drugged rats on the five "warm-up" shock-escape trials showed that mean latencies of treated and control groups did not differ significantly. This important observation suggests that drug effects on motor abilities could have played little or no part in the inhibition of performance on the test day seen in groups SB and BE. R. E. MILLER *et al.* (1957), presented data to indicate that in a dosage of 1.25 mg/kg suppression of the CAR by chlorpromazine could not be explained on the basis of motor effects.

A comparison to the data reported by MAY is of interest. He found that rats treated correspondingly to our controls (his "Experimentals",

group A) averaged 73% positive responses out of 25 trials each. Our finding of a practically identical 73.3% response (Control, 100 v., 30 trials) suggests comparability of the experiments in spite of differences in strain of rats and minor details of apparatus, procedure, and handling. It is then quite interesting to note that our chlorpromazine group BE averaged 9.3% positive trials which is again practically identical to the 9.0% performance of MAY's rats ("Controls", group A) which received *shock without buzzer* in phase two of training. Thus, the performance of group BE may reflect complete or nearly complete obliteration of phase two training.

Lastly, the test performances were analyzed to determine whether extinction was evident during the trials. The number of positive responses in the last third of the test trials was compared by the chi-square method to the expected value of one-third of the total assuming no extinction trend. Both of the control groups and group SB failed to show an extinction trend which was seen in groups SE and BE. Extinction was pronounced in the case of BE as only one out of 28 total responses was in the last third of the test, $P = 0.001$, but was also significant for SE with only 28 out of 117 responses in the last third, $P < 0.05$. Upon retesting on a subsequent day under the same conditions but without drug, there were no responses from group BE while responses were evoked from the other groups[1]. This gives further support to the observation of a more pronounced extinction effect in Group BE than in the other groups.

Discussion

The results show inhibitory effects of chlorpromazine on both acquisition and retention of a response motivated by conditioned fear. Little or no inhibition of response acquisition resulted when the drug was given on day one. That is, the conditioning of fear responses and "fear stimuli" to the barrier-crossing response (group SE) was not greatly hindered at this dosage of the drug. However, distinct inhibition of test performance was found when chlorpromazine was given during conditioning of pain-evoked fear responses and "fear stimuli" to the buzzer (group SB). Interference with this critical step in mediated generalization of the conditioned response is evident in the diminished conditioned-drive strength of the buzzer. The actual basis for interference cannot be determined from this experiment. The interference occurring here is one presumably occurring also in chlorpromazine inhibition of avoidance conditioning (LYNCH *et al.*). However, in that situation the formation of association between conditioned stimulus and unconditioned fear responses cannot be isolated for study as it can in this design. A residual direct effect of the drug on test day performance

[1] Exact figures not available.

from administration twenty-four hours previously is very unlikely since R. E. MILLER *et al.* (1957a) failed to find inhibition of test performance on the day following four consecutive days of treatment with this dose of chlorpromazine. The distinctive significance of this portion of the study is the demonstration that chlorpromazine in a low dosage could inhibit the transfer of a fear-motivated response to another stimulus by interference with the mediating or cue function of fear responses. Previous studies have not shown this effect of the drug on the mediating function of fear in a learning situation.

The greatest degree of performance inhibition occurred with administration on the test day. The treatment appeared to block completely the manifestation of the effects of "fear stimuli"-to-buzzer conditioning. The rats responded to the buzzer at the very low level to be expected if such conditioning had not been conducted. Extinction of even this slight response tendency occurred rapidly. Absence of spontaneous recovery on retesting indicates a persistent influence on extinction which may be especially significant. These effects are comparable to those reported in the CAR experiments of R. E. MILLER *et al.* (1957, 1957a). In the latter study, chlorpromazine was found to promote persistent extinction of an established CAR when given during four consecutive days of testing on an extinction basis.

R. E. MILLER *et al.* (1957a) interpret the enhancement of CAR extinction by chlorpromazine as learning during drug treatment — the learning of a new significance for the conditioned stimulus. They suggest that in the clinical application of chlorpromazine long-term therapeutic benefits may occur as pharmacological fear and anxiety reduction permits learning of new responses to displace the unadaptive responses characteristic of neurotic behavior.

In the present experiment chlorpromazine was found to produce persistent enhancement of extinction of a fear-motivated response. Moreover, drug treatment inhibited transfer of a conditioned response to a new eliciting stimulus when the transfer was mediated by conditioned fear. In these two effects distinct significance for the psychotherapeutic situation may be seen. As R. E. MILLER *et al.* (1957a) have previously suggested, the enhanced extinction of behavior associated with fear and anxiety should significantly promote therapy of neurotic behavior. This action might be particularly fruitful in combination with therapeutic procedures based on counter-conditioning processes or "behavior therapy" as proposed by EYSENCK, WOLPE, and others. In addition WOLPE (p. 98) has emphasized the psychological importance of mediated or secondary generalization (as well as primary stimulus generalization) of conditioned fear-motivated responses. He described this as a process by which neurotic behavior and symptomatology may progressively change for the

worse. Having demonstrated an inhibitory effect of chlorpromazine on a model of such secondary generalization of fear-motivated behavior, we suggest that this drug may prevent aggravation or "spreading" of the neurotic manifestations while acting simultaneously to promote the success of psychotherapy.

Summary

A procedure for determining the effects of drug treatment on fear-motivated behavior is presented. Although basically similar to the conditioned avoidance response (CAR), this procedure separates training events so that drug effects on the conditioning process can be partially analyzed. An avoidance-escape type of response is established efficiently with minimal training. The response shows a sensitivity to inhibition by chlorpromazine comparable to that of the conventional CAR.

Results of two portions of the experiment may indicate a psychological basis for reports of synergism between psychotherapy and chlorpromazine therapy. Chlorpromazine inhibited the establishment of an association between a conditioned stimulus and the internal fear state and its drive and cue functions. This inhibition constituted a hindrance to transfer or secondary generalization of the fear-motivated response to another set of circumstances. A therapeutic implication of this effect would be the prevention during chlorpromazine therapy of progressive extension of psychoneurotic symptomatology through secondary generalization. Chlorpromazine also promoted persistent extinction of the fear-conditioned response. This effect might have therapeutic value in promoting the removal of unadaptive responses and the restoration of behavior appropriate to the circumstances.

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Attempted Correlations between Behavioural and Biochemical Changes in Rats Following Reserpine and Chronic Administration of Amine-oxidase Inhibitors

By

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With 8 Figures in the Text

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Introduction

The daily administration of the amine-oxidase inhibitor Iproniazid to man has been reported to cause psychotic episodes (Ogilvie 1955), and this compound and similar substances have been examined as therapeutic agents for depressive illnesses (Truitt 1958, West and Dally 1959). This paper describes studies of the effects of the chronic administration of Iproniazid and related drugs to rats.

During the period in which the drugs were administered, the general behaviour of the rats was observed and measures of their performance at a behavioural task recorded daily. Constituents of the animals' urine were examined in an attempt to relate any changes in aromatic, particularly amine, metabolism with their behaviour. Reserpine was also studied because of its known amine-releasing properties (Shore *et al.* 1957).

Materials[1]

Male albino rats initially weighing 60—80 g from the Oxford University Biochemistry Department's stock were used throughout.

The amine-oxidase inhibitors employed were Iproniazid (*iso*nicotinoyl-*iso*propylhydrazine), Niamid (Nialamid 1-[2-benzylcarbamyl)-ethyl]-2-*iso*nicotinoylhydrazine), Isoniazid (*iso*nicotinoylhydrazine) and T.M. 6 (2-(4-methylphenoxy)-ethyltrimethylammonium bromide). Of these, Isoniazid has been shown not to inhibit amine-oxidase *in vivo* (Zeller *et al.* 1952).

T.M. 6 was prepared by a literature method (Goldfarb 1941), and the *iso*nicotinuric acid used as a chromatographic standard was prepared from *iso*nicotinoylhydrazine (Fox and Field 1943).

Experimental procedure

Measurement of behaviour. The specific item of behaviour measured was the time taken to climb 135 cm of vertical hemp rope in a modification of Winter's rope-climbing test (Winter and Flataker 1951).

[1] The authors are indebted to Roche Products Ltd., for the Iproniazid and Isoniazid, to Dr. H. H. R. Reinert of Pfizer Ltd., for the Niamid, and to Ciba Ltd., for the Reserpine.

The changes consisted of the exclusion of an electrified grid on the starting platform and the use of brief periods of high frequency white noise during training (WINTER, private communication). The rats were subjected to no fear provoking stimuli other than those unavoidably present in the unnatural training and test situations. A shorter climbing distance was used, 135 cm compared with 172 cm, as the animals were smaller than those of WINTER and FLATAKER. Throughout the training and experimental periods general observations of the behaviour of the rats before, during, and after testing were regularly made.

The apparatus consisted of two horizontal platforms 35 cm square, the upper of which was 135 cm above the lower. A vertical hemp rope 1.25 cm in diameter passed through a small hole in the centre of the lower platform and through a 17 cm diameter hole in the centre of the upper. A wooden cross-piece spliced through the rope bridged the upper aperture and acted as a foothold for the rats.

The animals were fed immediately after testing each day at 18.30 hr on 7.5 g of diet "41 B" (JOSEPH RANK Ltd.). Water was given *ad lib.*, and between Saturday and Sunday evenings food was also provided *ad lib.* The rats were kept in a dark-room in which lights were controlled by a time-switch to give daylight from 5.00 to 17.00 hr and an artificial dusk from 17.00 to 18.00 hr after which testing was begun.

A small amount of extra food was placed on the upper platform and an animal was trained to climb the rope spontaneously when placed on the lower platform. The time between its hind legs leaving the lower platform and its nose appearing above the level of the upper was measured to the nearest half second. Each rat made four climbs every evening and training was continued for 14 days or until the mean time of each group of rats lay within the range of one second for five consecutive days.

Design of experiments. Unless otherwise stated, a group consisted of four rats, and groups in the same series were studied simultaneously. The following experiments were carried out:

Series 1 Group A: Iproniazid
Series 2 Group A: Iproniazid
Series 3 Group A: Control given saline injections
Group B: Isoniazid
Series 4 Group A: Control
Group B: Niamid
Group C: T.M. 6
Series 5 Group A: Niamid
Group B: Iproniazid
Group C: Control
Series 6 Group A: Iproniazid and Reserpine in combination
Series 7 Group A: Iproniazid and Reserpine in combination
Group B: Iproniazid and Reserpine in combination
Group C: Control
Group D: Reserpine alone (2 rats)

Series 8 Group A: Reserpine alone (2 untrained rats)
Group B: Reserpine alone (2 untrained rats)
Group C: Reserpine alone (2 untrained rats)
Group D: Control (2 untrained rats)
Group E: Control (2 untrained rats)

Representative results are shown graphically in the appropriate section.

Dosage. Iproniazid, Isoniazid and Niamid were injected daily at 12.00 hr except on Sundays for periods of four weeks. They were dissolved in normal saline and given at the rate of 100 mg/kg body weight each day. The volume injected was 0.5 ml and was given subcutaneously alternately on the right and left sides of the animal's body. The control group receiving saline injections was treated in the same way.

T.M. 6 was given in a like manner for a period of eight days but at the rate of 200 mg/kg/day. Reserpine was administered subcutaneously at 16.00 hr in single doses of 5 mg/kg and the volume injected was 0.5 ml.

Chromatographic examination of urine. Pooled urine samples from each group were examined during the periods when their climbing performances were being tested. The short term experiments with groups of untrained rats were carried out in a similar manner.

Collection of samples. After the rat's climbing performances had been tested each evening at 18.30 hr the animals, in groups, were given the standard amount of food, but no water. At 21.00 hr when all the food had been eaten, the rats were placed in all-glass metabolism cages, given water, and the urine voided over the following twelve hours collected.

Extraction procedure. Urine samples from rats in the same group were combined, acidified (2N HCl) to pH 1 (B.D.H. universal indicator paper) and shaken for five minutes with charcoal (0.25 g per rat contributing to the sample) deactivated with 8% w/w n-octadecylamine (ASATOOR and DALGLIESH 1955). The charcoal, carrying adsorbed materials, was collected in a sintered glass filter and freed from inorganic salts by washing with water (5×20 ml). Adsorbed compounds were then eluted from the charcoal by washing with water containing 7% phenol (3×10 ml), and the resulting extract distilled at 17 mm from a bath at 45° with addition of water until the distillate gave no colour with ferric chloride, the residue finally being reduced to 1 ml.

Chromatographic procedure. Portions of this extract were applied to 23 cm square sheets of Whatman No. 1 chromatography papers, the applications being made, respectively, at the rate of 0.05 ml and 0.1 ml of concentrate from four rats, appropriate allowance being made when the number of rats in the group was less than four. The papers were run in the machine direction in ascending *iso*-propanol: ammonia (d 0.88): water (8:1:1, by volume, solvent A) for seven hours, dried, and then run

in the second direction in ascending n-butanol:acetic acid: water (4:1:1, solvent B) for five hours. Other solvent systems employed to assist in identifications were benzene: propionic acid: water (100:75:5, solvent C) and 20% aqueous potassium chloride (solvent D) (ARMSTRONG *et al.* 1958).

Examination of chromatograms. As soon as they were dry after the final run, the chromatograms were examined under a "Hanovia" lamp emitting 90% of its radiation at 2537 Å, and fluorescent, phosphorescent, and dark areas marked.

The papers carrying the smaller quantities of extract were then sprayed with ALTMAN'S reagent (4 g *p*-dimethylaminobenzaldehyde in 100 ml acetic anhydride) and then heated to 150° in air for 2 minutes. This treatment gives colours with hippuric acids (ACHESON *et al.* 1960).

The more heavily loaded papers were sprayed with EHRLICH'S reagent (1 g *p*-dimethylaminobenzaldehyde in 100 ml 2N HCl) which gives purple or blue colours with indoles. Immediately after spraying, these papers were placed on a light box and the coloured areas marked in as they developed. Whenever possible, control and experimental chromatograms were sprayed and examined simultaneously so that a direct comparison could be made. The intensities of the tryptophane, tryptamine and 5-hydroxyindoleacetic acid colours were compared with those on standard papers bearing known amounts of these materials so that a rough estimate could be made of the quantities present on the chromatograms.

A diazotized sulphanilic acid spray reagent which will detect 4-hydroxy-3-methoxymandelic acid on paper chromatograms prepared from human urine (ACHESON and DEARNALEY 1960) detected no phenolic materials when chromatograms were run bearing 0.10 ml of concentrate (from a four rat pool), but when 0.15 ml was used bad streaking occurred.

Results

Observations of behaviour. During training, all rats showed gradually diminishing fear responses followed by exploration of the environment with investigatory sniffing, and a progressive concentration on the rope until the habit of climbing for the food reward was so well established that other responses were almost entirely eliminated.

The more important experimental observations are set out systematically in Table 1. These changes in behaviour and physiological symptoms are described in greater detail below.

1) Iproniazid. The effects of Iproniazid were maximal during the first and second weeks of the experiments. The total behaviour pattern was altered and the climbing habit disorganised. Both abnormally rigid and abnormally active behaviour were observed. Rigidity took any of the

Table 1. *Symptoms observed when drug effects were maximal*

	Iproniazid	Niamid	Isoniazid	Reserpine
Tail reflex	Curls up (Straub tail)	Normal	Normal	Curls down
Grasp reflex	Absent	Absent	Absent	Present
Eyes	Wide	Wide	Normal	Closed
Piloerection	Absent	Absent	Absent	Present
Tremor	Absent	Absent	Absent	Present
Diarrhoea	Absent	Absent	Absent	Present
Posture	Normal	Abnormal	Normal	Abnormal
Spontaneous activity	Slight decrease	Decrease	Normal	Absent
Response to stimuli	Excessive	Excessive	Normal	Minimal
Aggression	Usually present	Present	Normal	Absent

following forms: crouching motionless for several minutes on the lower platform, stopping immobile part way up the rope, staring fixedly, and sitting motionless before the food on the upper platform. Hyperactivity was observed in biting the rope, climbing up and down, and rapid running without investigatory sniffing even, at times, over the food.

The Iproniazid treated animals were hypersensitive to external stimuli as shown in their reaction to noise and their wildness and aggression when handled. They exhibited a tendency to STRAUB's tail sign (STRAUB 1911) their eyes were protuberant, and when undisturbed they were less active, but their posture remained normal.

In the third and fourth weeks of the experiments, the climbing habit was re-established and the animals could be handled more easily, but felt slightly flaccid by comparison with the controls. In the third week of one experiment, however, one rat became acutely hyperactive and aggressive for two days, but recovered even though drug administration was continued.

On cessation of the drug treatment, the behaviour of all the animals became normal within 48 hours.

2) Niamid. Like Iproniazid, Niamid produced its greatest effects in the first two weeks of the experiments, but the onset of the symptoms was more rapid and reached a maximum within 24 hours; the observed phenomena were more dramatic, and the climbing habit was completely disrupted.

The rats walked with their bellies touching the ground, their legs were widely spread and the digits fanned. They appeared unable to grasp the rope and were therefore untestable. If placed on the hand palm upwards, they slid off when it was turned slowly through 90°. The effects gradually decreased during the following 48 hours of treatment.

They were hypersensitive to noise and showed excessive startle reaction, but unlike the Iproniazid treated rats they tended to move back-

wards when startled, sometimes falling off the testing platform. Backward movements, which were often circular, also occurred spontaneously as if the animals were retreating from something. A similar phenomenon has been observed in monkeys treated with lysergic acid diethylamide (COLE, unpublished observation).

The Niamid treated rats urinated when disturbed, as did those give Reserpine (see below). Aggression and excessive fear were seen when they were handled, but in general they were less active than control animals and their tails were normal. A tendency to photophobia was observed.

During the third and fourth weeks behaviour became more nearly normal, but, as in the case of Iproniazid, one rat became extremely aggressive and hyperactive in the third week of an experiment, and died, apparently of exhaustion, after three days, of these symptoms.

Almost without exception, small sores and scabs developed at the sites of injection about the beginning of the third week. When the drug treatment ceased, these healed completely, and the behaviour returned to normal after about four days.

3) T.M. 6. The group given T.M. 6 showed a slight impairment in climbing performance for three days and then an improvement. The experiment had to be discontinued after eight days owing to the development of large open sores at the site of injection.

No behavioural changes apart from some lethargy were noted.

4) Isoniazid. No marked changes occurred in this group.

5) Controls. The group receiving saline injections differed in no way from the untreated controls which showed neither behavioural nor physiological disturbances during the course of the experiments.

6) Reserpine. The syndrome produced in rats by Reserpine has been reported more fully (COLE and DEARNALEY 1960) and can be briefly described here as a strongly down curling tail, the opposite of that seen after morphine, a strong grasp reflex, closed eyes, piloerection, tremors, diarrhoea, hunched posture and greatly decreased activity. To these signs should be added a tendency to urinate when disturbed.

7) Iproniazid plus Reserpine (early). A group of 4 rats receiving Iproniazid in the usual way was given 5 mg/kg of Reserpine at 16.00 hr the second day of Iproniazid treatment. Three hours later the animals displayed agitated, confused and aggressive behaviour, refused food and were untestable. At 22.00 hr the only typical sign of Reserpine treatment was a slight tremor in one rat. The following day, ptosis, tremors, piloerection, hunched posture and marked lethargy were observed, but the grasp reflex and diarrhoea were absent, and the tails were normal. The same evening three of the animals were testable: the untestable animal dying the following day. A similar fatality occurred in a second, parallel group.

8) Iproniazid plus Reserpine (late). A group given Reserpine (5 mg/kg) in the fourth week of Iproniazid treatment showed no behavioural changes apart from some lethargy, slight ptosis and piloerection, and a tendency to assume the hunched posture. An increase in climbing time was observed, but no fatality occurred.

The experimental animals gained weight only slowly during the training and testing periods, but grew rapidly afterwards when given food *ad lib.* A decrease in weight occurred for one week in the group given Reserpine alone. This loss was not observed when Reserpine was given to the animals receiving Iproniazid.

Measurement of behaviour. The measures recorded enable the behaviour of each rat to be studied day by day and allow the significance of the observations to be assessed by complex analysis of variance. For the purposes of this paper, however, a detailed analysis was considered unnecessary and the results described are based on the mean and standard deviations of the time to climb the rope calculated for each group of rats for each day on which they were tested. It should be noted that the variance of these measures includes both the variation between rats and within rats. These two sources of variation were, however, of the same order and an analysis of a sample of the data showed that they did not differ significantly at the one per cent level. It was, therefore, considered reasonable to consider each of the times recorded as an independent measure of behaviour and when a comparison of the performance of two groups on the same day was possible the significance of the difference of the means was assessed by the usual "t" test for the significance of the difference between uncorrelated means. In general, a significance level of 0.001 was adopted because of the large number of possible comparisons in each experiment. Typical results are shown in Figs. 1 to 5.

The gaps at the end of each six day period of testing are Sundays when the rats were receiving food *ad lib.* and were not tested. The missing tests on Fig. 1 (day 12) and Fig. 5 (Group B, day —1) arise because the rats escaped and obtained food, thus invalidating the performance records of those days.

The number of readings taken each day (N) is given on the figures (e. g. $N = 16$ for a group of 4 rats), and on occasions when a single rat became untestable, the lower value of N is given for the relevant period.

1) Iproniazid. A record of the performance of a group of four rats given daily injections of Iproniazid is shown in Fig. 1. The vertical lines through the points on the curve represent the standard deviations of the sixteen daily measures whose means are plotted.

Considerable impairment in performance is evident during the first two weeks, followed by marked improvement in the third and fourth

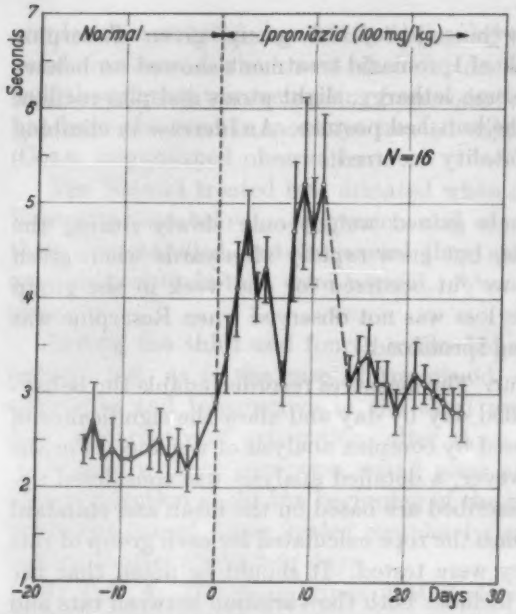

Fig. 1. Effect of Iproniazid on climbing rats. Means and standard deviations of times to climb rope on successive days

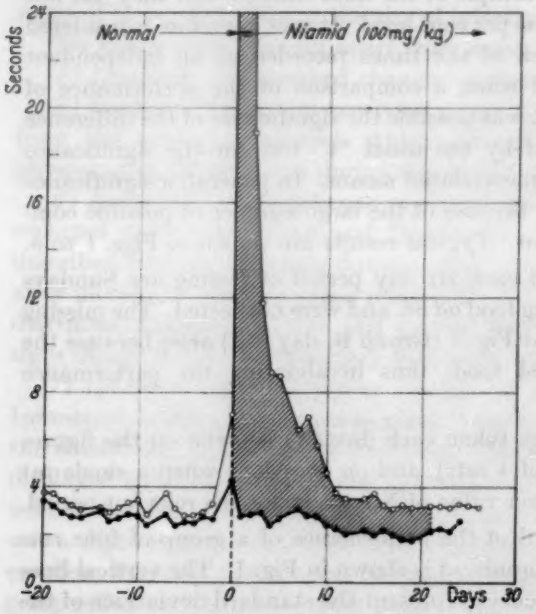

Fig. 2. Effect of Niamid on climbing rats: comparison with saline control. Mean times to climb rope on successive days. •—• Control group (N = 16), ○—○ experimental group (N = 16) ▨ indicates difference between corresponding means significant at 0.001 level

week. This sequence was reproduced in the two other experiments with Iproniazid.

2) Niamid. Fig. 2 shows the mean performance of two groups of four rats receiving daily Niamid and saline injections respectively. The shaded area between the curves indicates where the differences between mean performances on the same day were significant at the 0.001 level.

As with Iproniazid, the maximum effect was during the first two weeks followed by improved performance in the third and fourth weeks. Points of contrast are the more sudden and dramatic change during the first week on Niamid and a much more rapid and steady improvement during the second. At the end of the fourth week the performance on Niamid did not differ significantly from the saline control.

These results were confirmed in the second Niamid experiment.

3) T.M. 6. Owing to the short test period and the occurrence of the massive sores no reliable test results were obtained from the T.M. 6 treated rats.

4) Isoniazid. The mean performance of a group of rats receiving daily injections of Isoniazid is compared with a control group in Fig. 3 in the same manner as the Niamid and saline control groups, except that the shaded areas represent differences between corresponding means significant at the 0.05 level. Only at this level is the performance of the Isoniazid treated group distinguishable from that of the control, but it suggests a slight impairment of performance which in contrast to the Iproniazid and Niamid results, extends uniformly over the four week experimental period.

5) Reserpine. The effect of a single dose of Reserpine on a pair of otherwise untreated animals is shown in Fig. 4. The animals were untestable for two days but on the third day the mean performance did not differ significantly from that of the control group.

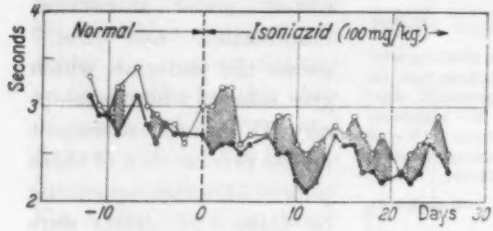

Fig. 3

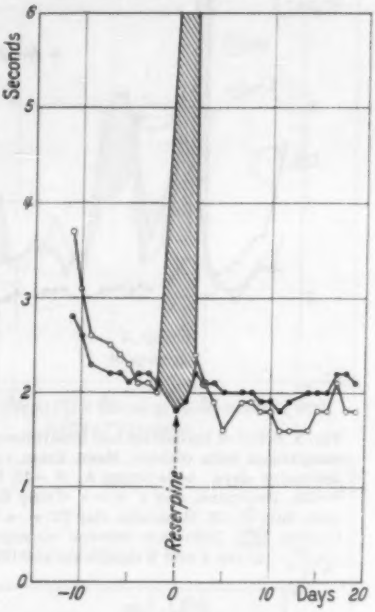

Fig. 4

Fig. 3. Effect of Isoniazid on climbing rats: comparison with control. Mean times to climb rope on successive days. •—• Control group (N = 16), ○—○ experimental group (W = 16), ▨ indicates difference between corresponding means significant at 0.05 level

Fig. 4. Effect of Reserpine on climbing rats: comparison with control. Mean times to climb rope on successive days. •—• Control group (N = 16), ○—○ experimental group: (N = 8). Reserpine given one day. No difference between corresponding means achieves significance at 0.001 level. ▨ Experimental group untestable

6) Iproniazid and Reserpine. Fig. 5 shows the effect of a single dose of Reserpine given during the Iproniazid treatment.

A group (A) of three rats given Reserpine on the second day of Iproniazid treatment was untestable for one day after which its mean performance approximated to the effect of Iproniazid alone but was signifficantly better at the 0.001 level for six of the following nine days.

A group (B) of four rats given a single dose of Reserpine on the 23rd day of Iproniazid treatment did not become untestable but showed an impairment of performance for two days which differed significantly at

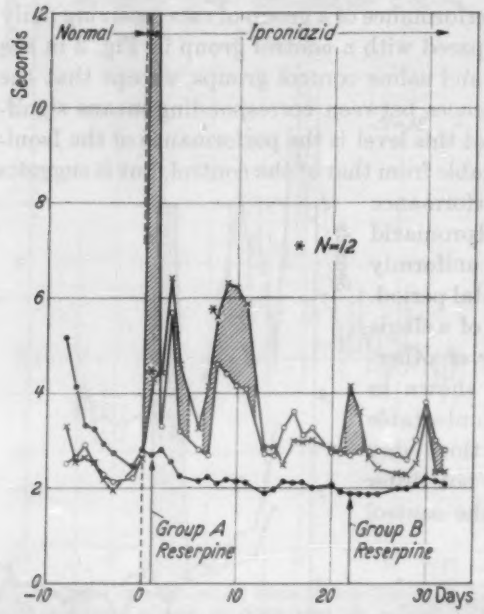

Fig. 5. Effect of Iproniazid and Reserpine on climbing rats: comparison with control. Mean times to climb rope on successive days. o—o Group A, $N = 12$ Iproniazid, days 0—32, Reserpine, day 1 ×—× Group B, $N = 16$ Iproniazid, days 0—32, Reserpine, day 22; •—• Group C, $N = 16$ Control. ▨ Difference between corresponding means of group A and B significant at 0.001 level

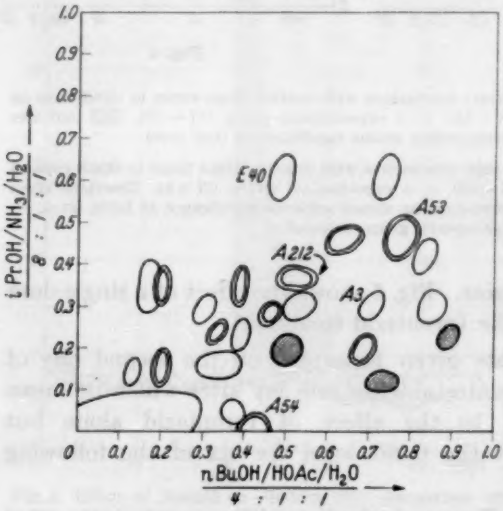

Fig. 6. Approximate Rf values and characterisation of spots detected under ultraviolet illumination. ○ Fluorescent spot. ◉ Dark spot. ◉ Phosphorescent spot

the 0.001 level from the mean performance of the group which had been given an early dose of Reserpine.

Changes in aromatic excretion pattern. The compounds located on two-dimensional paper chromatograms prepared and examined as described above are depicted in Figs. 6, 7 and 8. They are presented in a form which allows a ready comparison with published data (see references below).

Fig. 6 shows the characteristics of compounds detected under ultraviolet illumination and Fig. 7 shows the materials which give colours when developed with ALTMAN'S reagent on the reverse side of these papers. Materials appearing on these two charts were tentatively identified with reference to published work on human urine (ARMSTRONG *et. al.* 1956, and ACHESON and DEARNALEY 1960), except for *iso*nicotinuric acid whose identity was checked by observing the increased intensity of the colour on test chromatograms of extracts to which authentic material had been added. The identification numbers given are those used by ACHESON and DEARNALEY, but prefixed with the letter "A". Compounds

observed under ultra-violet light are only numbered if they correspond apparently to materials giving colours with ALTMAN's or EHRLICH's reagents.

The EHRLICH positive materials shown in Fig. 8 were identified by their Rf values and colour reactions with reference to published work on human urine (ARMSTRONG *et al.* 1958, and LEYTON 1958), but in addition, the positions of tryptophane, 5-hydroxyindoleacetic acid and indoleacetic acid were checked as in the case of *iso*nicotinuric acid. The presence of tryptamine was confirmed in the same way in all four solvent systems, and also by a positive response to a ninhydrin reagent claimed to be specific for this class of compound (JEPSON and STEVENS 1953). The identification numbers given in Fig. 8 are based on those used by ARMSTRONG, but prefixed with the letter "E".

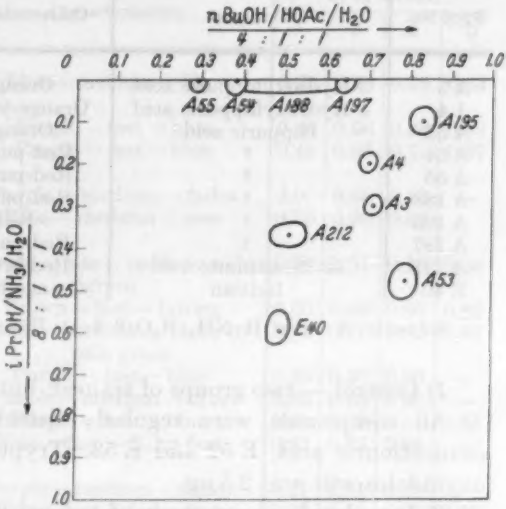

Fig. 7. Approximate Rf values of spots detected with ALTMAN's reagent

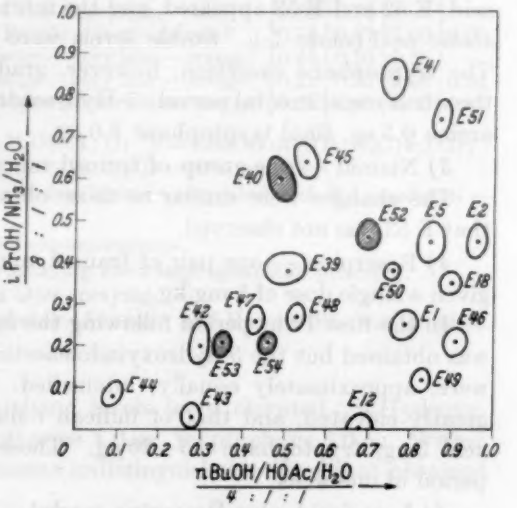

Fig. 8. Approximate Rf values of spots detected with EHRLICH's reagent; ● Brown or orange spot. ○ Purple or blue spot

Tables 2 and 3 give Rf values, tentative identifications and colour characteristics of the substances shown in Fig. 7 aud 8 respectively.

The quantities of materials quoted below are estimates of the amounts actually present on individual paper chromatograms prepared as described under "Experimental Procedure". Fluctuations observed in the excretion pattern during the various experiments are conveniently described under the following headings.

Table 2. *Characterisations, tentative identifications and Rf. values of Altman positive spots*

Spot No.	Compound	Characterisation	Rf in Solvent	
			A	B
A 3	3-Hydroxyhippuric acid	Orange	0.30	0.71
A 4	4-Hydroxyhippuric acid	Orange-yellow	0.20	0.70
A 53	Hippuric acid	Orange	0.48	0,79
A 54	?	Red-purple	0.00	0.38
A 55	?	Red-purple	0.00	0.29
A 188	?	Red-purple	0.00	0.42—0.62
A 195	?	Red	0.10	0.83
A 197	?	Red-purple	0.00	0.64
A 212	*iso*-Nicotinuric acid	Red-brown	0.37	0.51
E 40	Indican	Orange-brown	0.60	0.49

Solvents: A = iPrOH : NH_3 : H_2O (8:1:1); B = nBuOH : AcOH : H_2O (4:1:1).

1) Control — two groups of trained, but untreated rats.

All compounds were regularly detected except for tryptamine, *iso*nicotinuric acid, E 52 and E 53. Tryptophane 1.0—1.5 μg, 5-Hydroxyindoleacetic acid 2.5 μg.

2) Iproniazid — one group of trained rats receiving 100 mg/kg/day.

Within 24 hours of the first injection tryptamine, *iso*nicotinuric acid, E 52 and E 53 appeared, and the intensity of the 5-hydroxyindoleacetic acid colour fell. Stable levels were reached within three days. The tryptophane excretion, however, gradually increased throughout the whole experimental period. 5-Hydroxyindoleacetic acid 0.5μg, tryptamine 0.5 μg, final tryptophane 3.0 μg.

3) Niamid — one group of trained rats receiving 100 mg/kg/day.

The changes were similar to those obtained with Iproniazid except that E 53 was not observed.

4) Reserpine — one pair of trained, and two pairs of untrained rats given a single dose of 5 mg/kg.

In the first 12 hr period following the injections, the normal pattern was obtained but the 5-hydroxyindoleacetic acid, E 42 and E 43 colours were, approximately equally, intensified. The tryptophane level was greatly elevated, and that of indican reduced. 5-Hydroxyindoleacetic acid 5 μg, tryptophane 15—20 μg. These reverted to normal over a period of one week.

5) Iproniazid plus Reserpine (early) — one group of trained rats receiving 100 mg/kg/day of Iproniazid and given 5 mg/kg of Reserpine on the second day of the experiment.

The compounds detected in samples taken in the overnight period immediately following the Reserpine administration differed from those obtained with Iproniazid alone only in that the tryptamine, 5-hydroxy-

Table 3. *Characterisations, tentative identifications and Rf. vaules of Ehrlich positive spots*

Spot No.	Compound	Characterisation	Rf in Solvent			
			A	B	C	D
E 1	5-Hydroxyindole-acetic acid	Blue — medium — blue	0.25	0.79	0.19	0.54
E 2	Indoleacetic acid	Purple — fast — blue	0.45	0.96	0.88	0.88
E 5	Indolylacetylglut-amine	Purple — fast — blue	0.45	0.85	0.37	0.69
E 12	?	Purple — medium — fades	0.00	0.68	0.10	—
E 18	Indole-3-carboxylic acid	Rose — medium — rose	0.35	0.90	0.82	—
E 39	Tryptophane	Purple — late — fades to pale green	0.38	0.51	0.13	0.56
E 40	Indican	Brown — fast — brown	0.60	0.49	0.00	0.62
E 41	Tryptamine	Purple — medium — fades to pale green	0.84	0.76	0.42	0.45
E 42	?	Purple — fast — blue	0.20	0.30	0.00	—
E 43	?	Purple — medium — blue	0.03	0.28	0.00	—
E 44	?	Purple — late — blue	0.08	0.09	—	0.69
E 45	Spot "Q" (LEYTON 1958)[4]	Blue — fast — fades fast	0.62	0.55	0.08	—
E 46	?	Purple — medium — fades	0.21	0.91	0.58	—
E 47	?	Blue — fast — fades fast	0.26	0.43	0.06	0.69
E 48	?	Blue — fast — fades[1] fast	0.27	0.53	—	—
E 49	?	Purple — medium — fades[1]	0.12	0.83	—	—
E 50	?	Rose — medium — rose[1]	0.38	0.76	—	—
E 51	?	Purple — fast — purple[2]	0.74	0.87	1.00	0.00
E 52	?	Orange — very late — orange[3]	0.46	0.70	0.50	—
E 53	?	Orange — very late — orange[3]	0.21	0.35	0.06	0.94
E 54	?	Red-brown—very late—orange[3]	0.21	0.46	0.00	0.82

Solvents: A = iPrOH:NH_3:H_2O(8:1:1); B = nBuOH:AcOH:H_2O(4:1:1); C = Benzene:Propionic acid:H_2O(100:75:5); D = 20% aq. KCl.

Colours: Initial colour — rate of development compared with E 1 (medium) — colour after 24 hours.

[1] Observed on less than 10% of chromatograms.

[2] Purple colour visible before spraying when large quantities present.

[3] Colour develops 2—4 weeks after spraying.

[4] Identified as 6-sulphatoxyskatole (ACHESON and HANDS, Biochim. biophys. Acta (1961, in press).

indoleacetic acid and tryptophane levels were elevated. 5-Hydroxy-indoleacetic acid 1.0 μg, tryptamine 1.0 μg, tryptophane 3.0 μg. Within one week the pattern had become indistinguishable from that obtained with Iproniazid alone.

6) Iproniazid plus Reserpine (late) — one group of rats receiving 100 mg/kg/day of Iproniazid and given 5 mg/kg of Reserpine on the 23rd day of the experiment.

The Reserpine injections produced no observable change in the excretion pattern prevailing under Iproniazid alone.

At no time was 5-hydroxytryptamine detected in the urine samples. Standard chromatograms bearing 5-hydroxytryptamine creatinine sulphate showed a colour (violet-medium-blue) at Rf 0.62 in solvent A and 0.52 in solvent B, visible at 0.5 μg. Experiments on standard solutions of this material at concentrations from 0.1 to 1.0 μg/ml showed that it was extracted by the charcoal procedure with an efficiency of approximately 75%, and that its threshold of detection was 10 μg per urine sample.

Discussion

The rise in urinary 5-hydroxyindoleacetic acid following Reserpine treatment is well documented (eg. SHORE *et al.* 1957). It is possible that the compounds E 42 and E 43 are the O-sulphates of 5-hydroxyindoleacetic acid and 5-hydroxytryptamine, but this could not be verified as authentic samples were not available. The presence of these sulphates in rats' urine following administration of 5-hydroxytryptamine has been reported (CHADWICK and WILKINSON 1960). An increase in their concentrations after Reserpine treatment would be consistent with the amine releasing properties of the drug. The fall in indican excretion suggests that Reserpine interferes with the "detoxication" or absorption of indoles, or with their production by the intestinal flora.

The increase in tryptophane excretion cannot be interpreted with certainty. It cannot reasonably be attributed to the amine releasing properties of Reserpine. Whilst it is possible that the drug prevents the microbial degradation of tryptophane, it might be expected that extra tryptophane, thus available in the gut, would be adsorbed and metabolised by the host in the usual way, particularly in animals kept on a low diet. It is possible that Reserpine interferes with the transport of tryptophane across cell membranes and this is being investigated further. It has been suggested (Leading Article 1960) that an abnormality of this kind may be involved in HARTNUP'S disease.

When Reserpine was given early in the Iproniazid treatment the "excitation" ascribed to very large quantities of free serotonin (BRODIE and SHORE 1957) was observed. The amine releasing action of the drug was recognisable in that the tryptamine and 5-hydroxyindoleacetic acid excretion rose. Noteworthy too is that the tryptophane excretion was elevated, thought not so markedly as when Reserpine was given alone.

The results of giving Reserpine in the fourth week of Iproniazid administration are in marked contrast to its effects when given early or alone. It caused no observable change in excretion pattern and relatively little in behaviour, which is consistent with the finding that Iproniazid will inhibit the amine releasing properties of Reserpine (GIARMAN and SCHANBERG 1959).

Amongst the amine oxidase inhibitors studied, only those which are reported to be active *in vivo* produced the characteristic sequence of behavioural changes involving initial disruption and subsequent re-establishment of the climbing habit. This recovery of performance is particularly notable, and it is of interest and possible significance that the *iso*nicotinic acid derivatives examined which are capable of producing it, are those which are of use in pharmaco-psychotherapy.

The patterns of ALTMAN and EHRLICH positive spots detected in the chromatography are closely similar to those of the hippuric acids (ACHESON and DEARNALEY 1960) and the indoles (LEYTON 1958) found in human urine. The changes that occurred in these patterns during administration of the inhibitors are in accord with the findings of other investigators. The decrease in 5-hydroxyindoleacetic acid and the increase in tryptamine excretion have been observed in mice treated with Iproniazid (SCHAYER *et al.* 1954) and in human beings treated with Isocarboxazid (GUNTER *et al.* 1960). *Iso*nicotinuric acid, which undoubtedly arises from "detoxication" of *iso*nicotinic acid derivatives, has been detected in the urine of tuberculosis patients under treatment with Isoniazid (CUTHBERTSON *et al.* 1953). The appearance of the unidentified spots E 52 and E 53 and the gradual rise in tryptophane excretion are, as far as the authors are aware, new observations.

By the nature of the visual method employed, the measurements of the intensities of the indole colours are only very approximate, but nevertheless they yield information of considerable interest. Immediately on administering an amine-oxidase inhibitor the amount of 5-hydroxyindoleacetic acid fell by some 80%. The quantity of 5-hydroxytryptamine equivalent to this diminution is of the order of 20 μg per rat group per twelve hour collection period. The experiments with standard solutions of 5-hydroxytryptamine creatinine sulphate showed that had this been excreted, it would have been detected. In so far as this suggests a build-up of 5-hydroxytryptamine in the system, it is in agreement with the observation that dosage of rats with Iproniazid causes an increase in the 5-hydroxytryptamine content of the brain (GIARMAN and SCHANBERG 1959).

A possible explanation, therefore, of the initial impairment of climbing performance and the accompanying behavioural changes is that they are the results of accumulations of 5-hydroxytryptamine, and, possibly, of other compounds as well. Unfortunately no information can be derived from these results concerning the actual sites of such accumulation, whether cerebral or peripheral. The behavioural phenomena did not correspond to the effects of Reserpine (see Table 1) but this would not necessarily be expected, for, the rate of inactivation of pharmacologically active amines quite apart from their availability, is

likely to be of paramount importance in determining overall behaviour. Furthermore, it does not follow that the resulting local concentrations of free amines would be in the same relative proportions as those brought about by Reserpine treatment.

The re-establishment of the habit, however, is more difficult to understand. A possible explanation is that the same process which is responsible for the reduction in time taken to climb the rope on successive days *before* the drug treatment is also responsible for the reduction in time taken to climb the rope *during* chronic administration of the drug. Although this cannot be rejected *a priori* it is simpler to assume that some mechanism comes into play which opposes the initial biochemical disturbances.

It cannot be argued that amine-oxidase activity recovered as the rate of excretion of 5-hydroxyindoleacetic acid remained depressed throughout the whole experimental period. Nor is it likely that sulphation of 5-hydroxytryptamine achieved relative importance. This has been suggested as an alternative means of "detoxication" (CHADWICK and WILKINSON 1960), but no substance having chromatographic properties corresponding to the published description of 5-hydroxytryptamine-O-sulphate was observed to undergo any marked concentration changes during the experiments.

It is remarkable, however, that the gradual increase in tryptophane excretion eventually attained a value which was roughly equivalent to the fall in 5-hydroxyindoleacetic acid.

Thus it could be supposed that the rate of formation of 5-hydroxytryptamine ultimately falls to a level which compensates for the reduction in its rate of removal, an equivalent amount of tryptophane being excreted unchanged. It would be surprising, though, if none of this newly available tryptophane were metabolised by the kynurenine route, especially as the liver enzymes involved in tryptophane degradation adapt rapidly to increased amounts of substrate (KNOX and MEHLER 1950).

An interpretation of this kind does not insist that the animals' behaviour shall become indistinguishable from the control's for the rate of inactivation of 5-hydroxytryptamine must remain depressed, even though its concentration may return to normal.

Summary

Concurrent observations were made on the rope-climbing performance and aromatic excretion pattern of male albino rats during drug treatment. Iproniazid, Niamid and Isoniazid were administered chronically and Reserpine was given in single doses, alone, and in combination with the Iproniazid.

1. Iproniazid and Niamid disturbed the climbing habit which later became re-established even though drug treatment was continued. Urinary concentrations of 5-hydroxyindoleacetic acid fell and of tryptamine rose immediately on giving the drugs, and tryptophane excretion gradually increased.

2. Isoniazid had little effect on climbing performance.

3. A study of T.M. 6 was abandoned when large sores developed at the site of injection.

4. Reserpine given alone disrupted climbing and potentiated excretion of 5-hydroxyindoleacetic acid, tryptophane and two unidentified Ehrlich positive materials.

5. When given on the second day of Iproniazid treatment, Reserpine also disrupted climbing and increased excretion of 5-hydroxyindoleacetic acid, tryptamine and tryptophane. When given on the 23rd day, the climbing performance was impaired but no change was observed in excretion pattern.

6. Some physiological and overall behaviour changes during the experiments are described.

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Letters to the Editor · Briefe an die Herausgeber · Lettres à l'éditeur

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Effect of Paspalum Scrobiculatum Extract on Acutely Disturbed Schizophrenic Patients

A Preliminary Report

By

V. R. Deo and N. K. Bhide

(Received April 4, 1961)

The tranquillizing effect and comparatively low toxicity of dried ethanol extract of the husk of Paspalum scrobiculatum grain in animal experiments (Bhide and Aimen) prompted us to undertake clinical trials. Although, short term effect of 20—70 mg of this extract per day per os on one of us (N.K.B.) and on hypertensive patients suggested tranquillizing and hypotensive actions, a more critical and objective study was considered possible on acutely disturbed schizophrenic patients.

The group of 15 hospitalized schizophrenic patients consisted of 9 males and 6 females, of average age — 32 years (range 22—50 years), average duration of illness — 24 months (range 2—60 month) and average stay in the hospital — 13 months (range 1—57 months). Eleven patients were treated before on chlorpromazine (with ECT in one), one on chlorpromazine and reserpine with ECT, and 3 on barbiturates and paraldehyde. No treatment was given for about 3 weeks before the present trial except, occasionally in some patients, paraldehyde which was also omitted during the trial period. The dried ethanol extract of P. scrobiculatum was mixed with a few drops of edible oil and emulsified in water with gum acacia. In this form, 2—4 mg of the extract was given orally per day in 2 divided doses and increased rapidly to 15 mg per day. All patients received the extract for 7 days only. On the 7th day they were assessed on various points as given below (Table).

The improvement was more on the points of excitement, dirty habits etc., but much less on those of disordered thought processes like delusion. The clinical change usually occurred by the third or the fourth day of treatment. Drowsiness developed in one patient. In 7 patients the condition relapsed completely and in 7, partially within 4 days after discontinuing the extract. Only one patient maintained the improvement.

The blood pressure fell in 7 of the 9 cases by an average value, 14/10 mm of mercury (range 0—22/0—24 mm of mercury). Pulse rate rose in 12 patients by an average of 16 per minute (range 6—32 per minute), the tachycardia being unrelated to hypotension in most of the cases.

Table

Assessment	No. of patients at the beginning of the treatment	No. of patients on the 7th day of treatment
General gross excitement	15	nil in 10, slight in 2, moderate in 3
Discarding clothes	13	0
Climbing wall and windows	2	0
Self-injury	2	0
Intermittent shouting	10	0
Dirty habits (micturition and defaecation)	11	2
Delusions	3	3
Irrelevant muttering	7	4
Abusiveness	6	2
Injuring other patients	2	0
Cooperation and desire to work	0	6
Irritability on interrogating	0	5

As the clinical condition improved within 4 days after starting the extract and mostly relapsed equally rapidly after discontinuing the same, the observed effects cannot be attributed to chance alone. Considering the type of clinical disorder, the short duration of treatment and the expected degree of improvement, double-blind technique was not thought necessary during this initial phase of trial. On the other hand clinical effects of neuropharmacological agents should be interpreted very cautiously. Thus the present results appear only to be encouragingly suggestive of the tranquillizing effect of the extract of P. scrobiculatum. Further work is in progress.

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Beispiel: Dews, P. B.: Studies on behavior. IV. Stimulant actions of methamphetamine. J. Pharmacol. exp. Ther. **122**, 137—147 (1958a).

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